



SPECIAL LISTING

CURRENT CANCER RESEARCH

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**CANCER DIAGNOSIS:
BIOCHEMICAL,
IMMUNOLOGICAL AND
CYTOLOGICAL STUDIES**

OCTOBER 1981

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service • National Institutes of Health •
National Cancer Institute

A SERVICE OF THE
INTERNATIONAL CANCER RESEARCH DATA BANK (ICRDB)

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This **SPECIAL LISTING** of Current Cancer Research Projects is a publication of the **INTERNATIONAL CANCER RESEARCH DATA BANK (ICRDB)** Program of the National Cancer Institute. Each **LISTING** contains descriptions of ongoing projects in one selected cancer research area. Approximately 55 areas are covered in separate **SPECIAL LISTINGS**. Each title of these **SPECIAL LISTINGS** is published annually.

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CURRENT CANCER RESEARCH

I. BIOCHEMICAL MARKERS IN CANCER DIAGNOSIS AND PROGNOSIS

A. ENZYME MARKERS

1. TRANSFERASE

1. TERMINAL TRANSFERASE AS A BIOLOGICAL MARKER FOR HUMAN LEUKEMIAS

Tsiapalis, C. M., Papamichael, M., Hellenic Anticancer Inst., Dept. of Biochemistry/Immunology, 171 Alexandra Ave., Athens 603, Greece

OBJECTIVE: Develop and adapt biochemical and immunological methods to measure terminal transferase in human leukemias. Such measurements may become useful in prognosis, diagnosis and during treatment and in remission of leukemic patients.

APPROACH: Determination of terminal transferase activity in the peripheral blood leukocytes and/or bone marrow of such patients.

2. TERMINAL TRANSFERASE - A PROGNOSTIC MARKER IN LEUKEMIA

Campbell, W. F., Smithson, W. A., Ritts, R. E., Votaw, M. L., State University & Community College System of Tennessee, East Tennessee State University, School of Medicine, Dept. of Microbiology, Johnson City, Tennessee, 37601, United States of America

The overall objective of the project is to distinguish and categorize individual cases of acute lymphocytic leukemia (ALL) in order that the most efficacious therapeutic modality may be selected and implemented. The proposed investigation would focus upon terminal deoxynucleotidyl transferase (TdT) patterns in E-positive and E-negative populations of peripheral and bone marrow leukemic cells. The results of our current work have led to the innovation of three experimentally testable hypotheses: (1) TdT determinations at diagnosis of ALL can predict eventual relapse or long-term remission, (2) Sequential TdT determinations during remission can predict imminent relapse and (3) TdT observations at relapse can demonstrate clones of proliferating cells which are resistant to standard forms of induction therapy. Should the proposed study prove any or all of the hypotheses, as our preliminary data suggest, the bases would be established for improved clinical management of the cases of ALL which do not experience long-term remission.

BIBLIOGRAPHIC REFERENCES: Campbell, W.F., W.A. Smithson, R.E. Ritts and G.S. Gilchrist. Terminal deoxynucleotidyl transferase: Potential aid in diagnosis and prognosis of acute lymphocytic leukemia. *Blood* 52: 242, 1979. Campbell, W.F., W.A. Smithson, R.E. Ritts and G.S. Gilchrist. Terminal deoxynucleotidyl transferase patterns may distinguish clones of proliferating cells in acute lymphocytic leukemia. In *Advances in Comparative Leukemia Research* (Yohn, Lapin and Blakeslee, Ed.) Elsevier North Holland Inc. p. 505, 1980.

3. BIOCHEMICAL MARKERS IN LEUKEMIAS

Srivastava, B. I., Rosowski, W., Minowada, J., Roswell Park Memorial Inst., Dept. of Experimental Therapeutics, 666 Elm St., Buffalo, New York, 14263, United States of America

The objectives of this work are: 1) to evaluate terminal transferase immunofluorescent test for the differential diagnosis of leukemias and for monitoring disease; 2) to purify, characterize and produce antisera against DNases and proteases which

are present in AML cells but show little or no activity in ALL cells.

Thus, proposed work could provide diagnostic tests for the differential diagnosis of leukemias and for monitoring disease leading to improvements in chemotherapeutics management and increased patient survival.

BIBLIOGRAPHIC REFERENCES: Srivastava, B.I.S., Khan, S.A., Minowada, J., Henderson, E.S. and Rakowski, I., Terminal deoxynucleotidyl transferase activity and blast cell characteristics in adult acute leukemias. *Leukemia Research* 4:209-215, 1980. Srivastava, B.I.S., Chan, J.Y.H. and Siddiqui, F.A., Affinity chromatography of terminal deoxynucleotidyl transferase from calf thymus and human leukemic cells on a solid-phase immunoadsorbent. *J. Biochem. and Biophys. Methods* 2:1-9, 1980.

4. TERMINAL DEOXYNUCLEOTIDYL TRANSFERASE (TdT) IN LEUKEMIA AND LYMPHOMA

Hoffbrand, A. V., Ganeshaguru, K., Llewellyn, P., University of London, Royal Free Hospital School of Medicine, Dept. of Haematology, 8 Hunter St., London WC1N 1BP, England, United Kingdom

OBJECTIVE: To determine the value of measurement of TdT in peripheral blood cells, bone marrow cells and tumor tissue as a diagnostic marker in leukemia and lymphomas. The enzyme will be assayed biochemically and the results related to morphology, cytochemistry, immunological markers and response to therapy. In addition, a fluorescent anti-TdT will be prepared and its value compared with biochemical assay.

APPROACH: Samples will be obtained from this hospital and from patients entered into the M.R.C. leukemia trials. Specific projects will be undertaken on selected groups of patients, e.g., blast transformation of chronic granulocytic leukemia, undifferentiated leukemia etc.

PROGRESS: Studies are well under way with the biochemical assay. Over 1000 samples have been tested and a number of publications are in print, or have been submitted for publication.

5. TERMINAL TRANSFERASE IN MAMMALIAN HEMOPOIETIC TISSUE

Coleman, M. S., Deibel, M. R., Riley, L., Slaw, M. F., University of Kentucky, School of Medicine, Dept. of Biochemistry, Limestone & Euclid, Lexington, Kentucky, 40506, United States of America

Terminal deoxynucleotidyl transferase (TdT) is a DNA polymerase which has an unusual tissue distribution. It is found only in thymus and bone marrow. The enzyme has been purified to homogeneity from human cells and extensively characterized. An understanding of the biological role of this enzyme in the development of the immune system is the ultimate aim of this project.

BIBLIOGRAPHIC REFERENCES: Deibel, M.R., Jr. and Coleman, M.S., Biochemical Properties of Purified Human Terminal Deoxynucleotidyltransferase *Journal of Biological Chemistry*, 255, 4206-4212 (1980). Vines, R.L., Coleman, M.S., and Hutton, J.J. Reappearance of Terminal Deoxynucleotidyl Transferase Containing Cells in Rat Bone Marrow Following Corticosteroid Administration *Blood*, 56, 501-509 (1980).

6. ESTABLISHMENT OF AN IMMUNOLOGICAL SERUM TEST FOR OVARIAN CANCER

Berger, E. G., Verdon, B., Universitat Bern, Med. Chem. Institut, CH3000 Bern 9, Switzerland

OBJECTIVE: Diagnosis and follow-up of ovarian cancer by serum galactosyltransferase measurements by ELISA.

APPROACH: Since the structure of human milk galactosyltransferase (EC 2.4.1.22) appears to be very similar to an enzyme present in malignant effusions, purified human milk galactosyltransferase is used to elicit specific antisera in rabbits. A competitive ELISA for antigen determination is developed by applying a phosphatase-galactosyltransferase conjugate. The sensitivity of the assay is in the order of magnitude of nanograms/ml.

PROGRESS: The purification procedure of the milk antigen outlined by Gerber et al. was extended to exclude contaminants present in trace amounts but strongly immunogenic. Monospecific antibodies were prepared on a galactosyltransferase-agarose column and used for immunohistochemical and cytochemical localization of antigen in HeLa cells. Further work is aimed at correlating enzyme activity with immunoreactive protein in sera and body fluids.

7. STUDY OF A CANCER-ASSOCIATED GALACTOSYLTRANSFERASE

Weiser, M. M., State University of New York, Health Science Center at Buffalo, School of Medicine, Dept. of Medicine, 3435 Main St., Buffalo, New York, 14214, United States of America

Galactosyltransferase activity has been identified with intracellular membrane, primarily the Golgi membrane fraction. Recent reports have suggested the presence of galactosyltransferase activity on the cell surface membrane. Galactosyltransferase activity has also been detected as a soluble enzyme in various body fluids, including human serum. We have recently reported the detection of a cancer-associated serum galactosyltransferase isoenzyme (PNAS 73: 1319, 1976). The isoenzyme (GT-II) is separated from the major normal serum galactosyltransferase isoenzyme (GT-I) by polyacrylamide electrophoresis and activity assayed from eluates of gel slices. GT-II was found in the serum of 43 of 58 patients with various types of cancer, in only 2 of 39 patients with various non-malignant disorders, and was not detected in the serum of 22 normal control subjects.

The project proposes to evaluate the clinical significance of GT-II as an indicator of cancer by delineating the types of tumors in which it is detected. In addition, a group of patients with colorectal carcinoma will be followed up serially in order to determine the prognostic value of GT-II and its use as a marker of therapeutic response. It has been previously shown in animal tumor models that GT-II comes from the tumor. Therefore, tumors and normal tissue from patients will be analyzed for the presence of GT-II in order to determine the source of GT-II activity in cancer patients. Finally, GT-II from serum and tumor will be purified, and used to prepare a radioimmunoassay so as to facilitate the assay of GT-II.

8. IMMUNE ASSAYS FOR ENZYMES AND ISOZYMES IN CANCER

Shaper, J. H., Johns Hopkins University, School of Medicine, Dept. of Oncology, 725 N. Wolfe St., Baltimore, Maryland, 21205, United States of America

OBJECTIVES: Evaluate the immunodiagnostic significance of serum levels of UDP-galactosyltransferase in human breast and ovarian carcinoma. Specifically: develop a sensitive and highly specific radioimmunoassay for human serum galactosyltransferase, systematically examining the efficacy of antiserum produced from bovine milk, normal human serum, and human ascitic fluid; determine if a cancer-associated isoenzyme of galactosyltransferase, chemically similar to the isoenzyme reported in serum, is present in human ascitic fluid; assess the immunological cross-reactivity or relatedness of galactosyltransferase isolated from the three sources listed above; and once the radioimmunoassay has been developed, conduct, after consultation with the Project Officer, a clinical study correlating serum UDP-galactosyltransferase levels with tumor burden, disease state, and response to therapy.

9. CONCURRENT MEASUREMENT OF UDP-GALACTOSYLTRANSFERASE AND CARCINOEMBRYONIC ANTIGEN LEVELS IN CANCER PATIENTS

Munjal, D. D., Hill, J. R., Court, D. A., Riverside Methodist Hospital, Medical Research Laboratory, 3535 Olentangy River Rd., Columbus, Ohio, 43214, United States of America

OBJECTIVE: a) To quantitate and evaluate the combined clinical usefulness of GT and CEA in cancer; b) to isolate and characterize GT from malignant material (pleural fluids and liver metastases); c) to produce anti-GT antisera against partially purified preparations of GT in rabbits, and evaluate their usefulness in development of an immunoassay for quantitation of GT in cancer serum.

APPROACH: a) The levels of GT in dialyzed malignant sera will be quantitated using NAD coupled spectrophotometric enzyme assay which uses N-acetylglucosamine as an acceptor. The levels of CEA will be measured by using an enzyme immunoassay (EIA). b) GT will be isolated from malignant material (effusions and tumor tissues) by extraction with ammonium sulfate (30-70%) followed by ion exchange and gel filtration chromatography. The rabbits will be immunized against partially purified GTs, and the antisera obtained will be absorbed against normal human tissues (colon and liver) and normal rabbit serum.

PROGRESS: In this study we quantitated and evaluated CEA and GT levels in 80 patients (45 cancer and 35 benign disorders) in paired samples. GT greater than 110 U/L and CEA greater than 5 ng/ml were taken as 'elevated.' When compared to values for normal subjects, statistically significant results were obtained for patients with cancer and benign disorders. Of the 45 cancer patients, 31 had localized disease and the remaining 14 a metastatic disease. Interestingly, 8 of the 14 metastatic cancer patients had CEA levels less than 5 ng/ml, while GT levels were very high indicating a discordance between CEA and GT measurements. CEA values greater than 5 ng/ml were, in general, observed in either patients with colorectal malignancies or with benign GI and liver disorders. Although these results indicate that the combined measurement of CEA and GT in proven malignancies provides a better detection potential than either CEA or GT alone, the immunochemical characterization of GT activity (since we are measuring total GT which comprises normal GT-I and cancer-associated GT-II) using absorbed rabbit antiserum prepared against purified GT from malignant material is essential and is being pursued. (Supported by Riverside Methodist Hospital Medical Research Foundation).

10. ADVANCED BREAST CANCER - A STUDY OF SERIAL PLASMA GLYCOSYLTRANSFERASE LEVELS IN PATIENTS BEING TREATED BY CHEMOTHERAPY

Rubens, R. D., Bulbrook, R. D., Kessel, D., Imperial Cancer Research Fund, Breast Cancer Unit, Lincoln's Inn Fields, London WC2A 3PX, England, United Kingdom

Levels of plasma glycosyltransferases appear to be elevated in neoplastic disease, although the relationship between enzyme levels and extent of disease has not yet been established. The level of plasma sialyl transferase has been found elevated in patients with advanced breast cancer and in animals bearing metastasising mammary tumors. This study is directed at assessing value of measuring plasma glycosyltransferases as an index of the extent of tumor load in patients with metastatic breast cancer receiving chemotherapy. These enzymes are being estimated before starting chemotherapy and on the first day of subsequent courses of chemotherapy in patients from the previous project to see if a positive correlation with clinical response can be found.

11. OAT-CELL CARCINOMA OF THE BRONCHUS - THE USE OF GLYCOSYLTRANSFERASES AS MARKERS

Sparrow, G. E., Rubens, R. D., Bulbrook, R. D., Imperial Cancer Research Fund, Medical Oncology Clinic, Lincoln's Inn Fields, London WC2A 3PX, England, United Kingdom

Glycoproteins are a major structural component of cell surfaces and their composition may alter during neoplastic transformation. One such alteration is an elevation in the level of sialic acid on the cell surface, implying an increase in the activity of the sialyltransferase enzyme. Such an increase has been found in breast and colon tumor tissue and in the plasma of patients with a variety of tumors. The purpose of this study is to measure the plasma sialyltransferase levels in patients with oat-cell carcinoma of the bronchus, both before and during treatment, to assess its value as a tumor marker.

12. GLYCOSYLTRANSFERASES AND THEIR ASSOCIATION WITH CANCER

Fraser, I. H., Mookerjee, S. S., Memorial University of Newfoundland, Dept. of Biochemistry, Health Science Complex, St. John's, Newfoundland, A1C 5S7, Canada

Serum glycosyltransferases are elevated in patients with cancer. The specificity of such changes is unclear as these enzymes also increase during inflammation and various liver diseases. Using isoelectric focusing techniques we propose to investigate the various isozymes of fucosyl-, sialyl- and galactosyl transferases in the serum of several animal models. Liver specific isozymes should increase in the serum after partial hepatectomy or in the inflammatory response after a subcutaneous injection of turpentine. Tumor specific isozymes are expected in the serum of rats with various hepatomas or mammary carcinoma. The effects of several anti-tumor agents such as methotrexate, 5-fluorouracil and cycthalasin B will also be studied. The ability to detect tumor specific glycosyltransferase enzymes would aid in the detection of cancer as well as in the monitoring of patients after surgery or chemotherapy.

13. EVALUATION OF THE ROLE OF BIOMARKERS IN TUMOR PROGRESSION

Nagarajan, B., Cancer Inst., Dept. of Microbiology, Adyar, Madras 600020, Madras, India

This project is an attempt to evolve methods for application of specific biological and biochemical markers in the clinical prognosis of tumors. A number of enzymes have recently been reported to undergo some kind of change either in total content and catalytic activity or in their isozyme pattern with the progression of tumor. Attempts are being made to correlate changes in serum and urine of cancer patients of enzymes like arginase ornithine carbamyl transferase and also arginine related metabolites like polyamines. Plans are underway to include a few more markers like terminal deoxynucleotidyl transferase, gamma-glutamyl transpeptidase.

Published work from this laboratory has been reviewed in *Biochemical Reviews (India)*, 50: 149-159 (1980).

2. POLYMERASES AND NUCLEASES

14. REVERSE TRANSCRIPTASE IN TUMOR CELL (HUMAN MILK, TUMORS)

Tsiapalis, C. M., Kottarides, S., Delinasios, J., Tranga, T., Hellenic Anticancer Inst., Dept. of Biochemistry/Virology, 171 Alexandrou Ave., Athens 603, Greece

OBJECTIVE: Develop and adapt methods of assay for reverse transcriptase (RT) in several human tumors of interest in Greece (i.e., breast, spleen, eye). Correlate these studies with results from morphological, pathological data.

APPROACH: Use cell cultures and fresh and/or frozen tumors or milk from genetically and geographically isolated populations in Greece. Use both biochemical and immunological assays for RT detection. Detection of RT in any of the above human material will be followed by the isolation and characterization of the enzyme.

15. HUMAN REVERSE TRANSCRIPTASE - A DIAGNOSTIC TEST

Marcus, S. L., Sloan Kettering Inst. for Cancer Research, 410 E. 68th St., New York, New York, 10021, United States of America

This is part of a broader project. A summary of this subproject is not available.

16. DETECTION OF EARLY PANCREATIC CANCER - ROLE OF RIBONUCLEASE, PANCREATIC ONCOFETAL ANTIGEN AND OTHER TUMOR-ASSOCIATED ANTIGENS (ABBREVIATED)

Tytgat, G. N., Teunen, A., Huijbregtse, K., de Vet, B., Hoek, F., Hoisma, H. F., Universiteit van Amsterdam, Dept. of Medicine, Spui 21, Amsterdam, Netherlands

OBJECTIVE: Evaluation of various new methods for detection of pancreatic cancer (screening for pancreatic function abnormalities with PABA test and Lundh test; determination of ribonuclease, pancreatic oncofetal antigen, CEA and lactoferrin, in serum and in pure pancreatic juice; analysis of endoscopic retrograde pancreatographic findings in various diseases of the pancreas; correlation of the findings with the final state of patients with pancreatic cancer).

APPROACH: a) Determination of ribonuclease, pancreatic oncofetal antigen and other tumor-associated antigens in the serum of 100 patients with various abdominal malignant and nonmalignant conditions and of 100 normal age- and sex-matched control subjects. b) Detailed study of 100 patients, referred for a suspicion of possible pancreatic malignancy by 1) analysis of pancreatic functions using the PABA test, Lundh test, secretin pancreozymin test, fecal fat excretion rate, etc.; 2) determination of ribonuclease, pancreatic oncofetal antigen, CEA, and lactoferrin in serum and pure pancreatic juice; 3) critical analysis of radiological findings on the pancreatic ductal system obtained by endoscopic retrograde cholangiopancreatography; 4) cytological examination of pure pancreatic juice for exfoliating malignant cells; and 5) correlation of all the above findings with the final diagnosis and condition of the patients with and without pancreatic cancer.

17. RIBONUCLEASE ACTIVITIES IN HUMAN SERA

Karpetsky, T. P., Levy, C. C., Wiernik, P., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Molecular Biology Laboratory, Baltimore, Maryland, 21201, United States of America

The members of seventeen families, comprising seventy-nine normal volunteers, having no clinical evidence of disease, were examined for multiple ribonuclease activities using polynucleotide-polyacrylamide gel electrophoresis. Gels (6.5%) were prepared containing polyadenylic acid (0.5%). Serum samples (15 microliters) were electrophoresed using conditions under which enzyme catalyzed hydrolysis of the polynucleotide did not occur (pH 8.6). After electrophoresis, gels were incubated and stained, revealing RNase activities as colorless bands in otherwise purple gels. The high resolution obtained with this technique enables 22 distinct activity bands to be visualized. Although no two individuals had identical activity patterns, a statistical profile of a normal serum pattern was obtained. These studies are the basis for examination of activity patterns of individuals having leukemia. Serum RNase patterns of patients change in response to treatment of disease. Coincident with treatment, certain enzymes appear or disappear, and the intensities of some bands undergo marked alterations.

BIBLIOGRAPHIC REFERENCES: Levy, C.C. and Karpetsky, T.P.: *Human Ribonucleases*. In Holcenberg, J. (Ed): *Enzymes as Drugs*. New York, Wiley and Sons, 1980, in press.

18. HUMAN PANCREATIC RIBONUCLEASE AND CANCER

Glitz, D. G., Weickmann, J., University of California, Los Angeles Campus, School of Medicine, Dept. of Biological Chemistry, 2025 Zonal Ave., Los Angeles, California, 90024, United States of America

Human pancreatic ribonuclease has been purified from tissue obtained at autopsy. The enzyme will be characterized in comparison with the well-studied bovine pancreatic RNase, with particular attention to enzyme mechanism and specificity, physical properties, and immunological relatedness. Antibodies directed against the human pancreatic enzyme will be used to quantitate cross-reacting protein in extracts of other human tissues, and to compare the structure of the enzyme to pancreatic ribonucleases of other organisms. A radioimmunoassay procedure will be used to measure circulating levels of pancreatic RNase in the serum of individuals with pancreatic cancer and related diseases. It is hoped that it will then be possible to resolve the dispute over the value of ribonuclease assays in the detection of pancreatic cancer, and perhaps to develop a practical and useful technique for the early detection of pancreatic carcinoma.

19. SERUM URINE AND CSF RNASES IN HEALTH AND DISEASE

Dekker, C. A., Blank, A., University of California, Berkeley Campus, School of Letters & Science, Dept. of Biochemistry, Berkeley, California, 94720, United States of America

Our specific objectives are: (1) To ascertain the fundamental physical nature of the multiple ribonucleases of human blood serum, urine and pancreas; (2) to characterize physical properties of the RNase of leukocytes and the 'acid RNase' of urine; (3) to characterize the RNases of normal cerebrospinal fluid and of cerebrospinal fluid showing elevated total RNase activity; (4) to test the hypothesis that the elevated RNase activity observed

in the plasma of cancer patients is due, at least in part, to increased concentration of RNase species normally present in plasma; (5) to determine if alterations in the level and/or electrophoretic profile of plasma RNases are correlated with fibrocystic disease of the breast and/or familial history of breast cancer; (6) to compare the level and electrophoretic distribution of plasma RNases before and after resection of malignant tumors and to determine whether the observed differences and/or post-operative level or distribution have prognostic significance; (7) to determine whether analysis of plasma RNase activity may be utilized in the follow-up care of patients; (8) to synthesize the phosphorothiolate analogs of dinucleoside monophosphates and to evaluate their utility as standard substrates for assay of plasma RNase activity.

Methods to be employed include standard techniques for purification of enzymes, e.g., column chromatography, and for determination of the physical properties of proteins, e.g., amino acid analysis. Plasma RNase levels of cancer patients will be measured in test tube assays using polymer substrates as well as the novel synthetic substrates. Polyacrylamide gel electrophoresis in conjunction with activity staining for RNase activity will be extensively employed in the fundamental biochemical studies and in the clinical studies.

20. MEASUREMENT OF 5-NUCLEOTIDASE IN SERUM

Persijn, J. P., Nederlands Kanker Instituut, Cancer Hospital, Antoni Van Leeuwenhoek, Ziekenhuis Plesmanlaan 121, Amsterdam, Netherlands

Available methods for the automated determination of serum nucleotidase (5N) usually require long incubation times, incubation at temperatures higher than 37 degrees C, or large sample volumes resulting in high blank values. Since difficulties are encountered under these circumstances, we decided to look for a better and more sensitive assay procedure. We also tried to maintain the advantages and clinical significance of our earlier method, i.e., the use of phenylphosphate for the inhibition of non-specific alkaline phosphatase.

Previous experiments based on the determination of released phosphate revealed that serum 5-nucleotidase (5N) hydrolyzes cytidine-5-monophosphate (CMP) at a much higher rate than adenosine-5-monophosphate (AMP), the substrate commonly used for the assay of 5N.

It seemed attractive to take advantage of these higher activities with CMP to obtain a simple and sensitive automated procedure for the measurement of 5N.

As a first step, a method was developed in which the cytidine liberated by the action of 5N is deaminated enzymatically and the ammonia thus released is measured with the Berthelot reaction. Deamination is performed by cytidine deaminase (CDA), the source of deaminase being a crude extract from *E. coli* cells. The CDA activity is not affected by CMP (7mM), or serum. Purification of the crude CDA was necessary to eliminate interfering enzymes. With a semi-purified CDA, 5N activities could be measured in sera at about twice the rate as compared to the reference method using AMP as a substrate. Further studies are in progress. This work is performed in collaboration with Dr. Van der Slik (Leiden).

3. PHOSPHATASES

21. MEASUREMENT OF LEUKOCYTIC ALKALINE PHOSPHATASE (LAP) LEVELS IN CANCER PATIENTS

Rana, R., Dirr, A., Rana, J., Nadal, N., Leone, B. A., Rabinovich, M. G., Arevalo, E. M., Hospital Regional Neuquen, Buenos Aires y Talero, 8300 Neuquen, Argentina

OBJECTIVES: a) To verify the increase of leukocytic alkaline phosphatase (LAP) in cancer patients as compared to the normal population. b) To determine whether statistically significant differences exist between patients with localized (30 to 50 pts) and with disseminated (30 to 50 pts) neoplasia. c) To correlate increases in LAP with various tumor locations and metastases.

MATERIAL, METHOD AND MEASUREMENT CRITERIA: We will measure LAP levels in normal subjects and in patients with solid neoplasias, independent of localization and clinical status.

The cytochemical method of Kaplow (1963) will be used, counting 100 neutrophilic granulocytes with a score of 0 to 4

according to the intensity of alkaline phosphatase activity manifested by cytoplasmic granules within the neutrophils.

We will study 90 to 130 patients and controls. (Translated)

22. ALKALINE PHOSPHATASE ISOENZYMES IN METASTATIC MALIGNANCY INVOLVING BONE

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The aim of this ongoing study is to measure the effect of osteolytic carcinoma metastases on the activity of the bone isoenzyme of alkaline phosphatase. This has been stimulated by the contention of several reviewers of the subject who claim that alkaline phosphatase activity is raised with cancer metastases to bone. Data on more than fifty patients with secondary bone neoplasia have been accumulated. Our studies indicate that raised alkaline phosphatase activity in metastatic cancer denotes liver metastases and that it is the activity of the liver isoenzyme of alkaline phosphatase which is raised while the bone isoenzyme remains normal. Attempts are being directed at obtaining a satisfactory measurable graphical representation of the multiple electrophoretic bands obtained in the gel. Bone specimens have been collected at autopsy from several deceased patients who had been studied in life. These specimens are to be examined histochemically for alkaline phosphatase activity and histologically for osteoblast activity in regions neighboring metastases.

This minor project, using polyacrylamide gel electrophoresis to delineate the isoenzymes of serum alkaline phosphatase, is continuing. These findings have obvious diagnostic, therapeutic and prognostic implications. The Oncology Division of the Johns Hopkins University School of Medicine and the Baltimore Cancer Research Center are collaborating on this study. There are no new results to report at this time.

23. CEA AND FHAP AS BREAST CANCER MANAGEMENT PARAMETERS

Torney, D. C., Larson, F. C., Crowley, J. J., Friedman, R. B., Entine, S. M., University of Wisconsin, Madison Campus, Medical School, Dept. of Human Oncology/Medicine, 450 N. Randall Ave., Madison, Wisconsin, 53706, United States of America

This project is designed to evaluate the relative effectiveness of two cancer-associated biochemical assays for providing decision making parameters in the clinical management of patients with breast carcinoma. The carcino-embryonic antigen (CEA) and serum fast homoarginine sensitive alkaline phosphatase (FHAP). The two assays will be evaluated individually and as a matrix to determine whether or not they provide clinically useful prognostic information preoperatively, postoperatively, or prior to treatment for metastatic disease. The role of serial determinations of CEA and FHAP levels, individually and as a matrix, for monitoring disease activity will also be investigated postoperatively, and in metastatic disease.

24. CARCINO-PLACENTAL ALKALINE PHOSPHATASE IN GYNECOLOGICAL TUMORS

Hajje, W. G., Baggerman, L., Talerman, A., Meerwaldt, J. H., Driel, J. V., Rotterdamsch Radiotherapeutisch Instituut, Dept. of Clinical Chemistry/Radiotherapy, Groene Hilledijk 301, Box 5201, 3075 EA Rotterdam, Netherlands

OBJECTIVE: To assess the usefulness of a specific and sensitive determination of CPAP in diagnosis and follow-up of patients with gynecological tumors.

With a sensitive solid phase EIA, CPAP is determined in sera of well documented patients with gynecological tumors under treatment in our institute and in cooperating gynecology departments of general hospitals in Rotterdam.

Histology of the tumors and CPAP-content of tumor tissue, as well as the role of other TM's (CEA) are evaluated together with the CPAP serum values in a longitudinal study.

25. PROSTATIC ACID PHOSPHATASES IN PROSTATIC CANCER (ABBREV)

Vihko, P., Vihko, R., Kontturi, M., Lukkarienen, O., Oulun Yliopisto, Dept. of Anatomy/Clinical Chemistry, Kajaanintie 52 A, 90220 Oulu, Finland

OBJECTIVES: To determine the usefulness of the radioimmunological determination of prostatic acid phosphatase 1

and II in the early diagnosis of prostatic carcinoma, in the follow-up of the course of the disease and in the evaluation of the effect of the various forms of treatment on the disease. To determine the cellular organelle specificity of prostatic acid phosphatase I and II, employing their specific antibodies.

APPROACH: Prostatic acid phosphatases are measured by radioimmunoassays, as described by P. Vihko et al., Clin. Chem. 24:1915, 1978. Immunocytochemistry is performed with the use of light and electron microscopy. PAP binding protein in the serum is purified by PAP affinity chromatography.

PROGRESS: The reference range for immunoreactive prostatic acid phosphatase has been determined (see ref. above). The follow-up of endocrine therapy (12 months): The radioimmunoassay of prostatic acid phosphatase showed clear advantages over the measurement of catalytic activity (P. Vihko, et al., Scand. J. Clin. Lab. Invest. 152:46, 1979). The prospective study concerned with the clarification of the possibilities of the radioimmunological determination of prostatic acid phosphatase in the early diagnosis of prostatic carcinoma is in progress.

26. RADIOIMMUNOLOGICAL DETERMINATION OF PAP AND ITS USE IN THE DIAGNOSIS AND CONTROL OF PROSTATIC CANCER PATIENTS (ABBREV)

Skinningsrud, A., Nustad, K., Radiumhospitalet, Central Laboratory, Montebello, Oslo 3, Norway

OBJECTIVES: The purpose of the project is to improve the biochemical methods for diagnosis and follow-up of prostate cancer patients.

APPROACH: 1. Prepare the results of the work that has already been done on purification of prostatic acid phosphatase (PAP) and evaluation of the clinical usefulness of prostatic acid phosphatase (PAP-RIA) in patients from one cancer hospital and from two urological departments. 2. Compare PAP-RIA to an immunoradiometric analysis of sera and bone marrow sera in a selected patient group. 3. Physicochemical characterization of purified PAP. 4. Physicochemical variation of PAP in cancer tissue and sera by means of isoelectric focusing, affinity electrophoresis against lectines followed by crossed immunoelectrophoresis, enzymatic staining or fluorescent antibody staining.

PROGRESS: Papers on the purification of PAP, the preparation of the R.I.A., and evaluation of patient results will probably appear in early 1980.

27. DIAGNOSTIC SPECIFICITY OF PROSTATIC ACID PHOSPHATASE - AN ANALYSIS OF IMMUNOHISTOCHEMISTRY AND RADIOIMMUNOASSAY

Manley, P. N., Queen's University, Dept. of Pathology, King St. W., Kingston, Ontario, K7L 3N6, Canada

Antisera raised by several investigators to prostatic acid phosphatase have shown much greater specificity than enzymatic assays for the diagnosis of prostatic adenocarcinoma. Nevertheless, PAP has been elevated in a variety of extra-prostatic neoplasms including pulmonary oat cell carcinoma and pancreatic carcinoma, and is present in the serum, urine, and vaginal fluid of the female. A thorough investigation of the specificity of tissue and serum immunologic techniques using PAP antisera has not been performed.

The objectives of the proposed investigation are: 1) to define the specificity of PAP antiserum in the male, 2) to establish whether PAP tissue immunoreactivity in the male is diagnostic of prostatic adenocarcinoma, and 3) in the female, to determine the cell(s) of origin of immunoreactive PAP; to test cancer(s) arising from the cell for PAP production; to correlate the levels of serum, vaginal, and urine PAP with variations in hormonal status.

Immunoassays for PAP are now being sold commercially. Clarification by this study of their diagnostic specificity in the male would increase their usefulness in the diagnosis of prostatic adenocarcinoma. Further definition of PAP in the female may show PAP to be a novel, highly specific marker for a hormonal-y responsive tumor of the female genitourinary tract.

28. RESEARCH PLAN FOR EARLY DETECTION OF PROSTATE CANCER

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The major objective of this research is to develop a biochemical laboratory test that will differentiate patients with prostate cancer from those free of this disease. Previous studies strongly support the concept that distinct isoenzyme patterns for acid phosphatase and leucine aminopeptidase are present in the prostatic fluid of patients suffering from prostate cancer as compared to patients who do not have this disease. The main thrust of this proposal is to develop a reliable and reproducible laboratory technique for the identification of acid phosphatase and leucine aminopeptidase isoenzymes in human prostatic fluid. Once the test is developed, it will be applied in analysis of the prostatic fluid from a set group of patients with biopsy proven prostate carcinoma and a matched number of patients free of this disease in two distinct age groups and tested for statistical significance. This new information would be an extremely useful tool for the detection of patients with early stage prostate cancer, especially those with otherwise clinically inapparent disease. The first phase of this research project has almost been completed. Successful separation of acid phosphatase into roughly ten to fifteen isoenzymes has been accomplished and awaits densitometric clarification. Leucine aminopeptidase isoenzyme separation has been accomplished but further development of technique will be necessary for sharper separation of the stained isoenzyme bands. Recent purchase of a recording densitometer should allow for uninterrupted transition into the clinical phase of this project in the next four to six weeks.

29. IMMUNOCHEMICAL STUDIES OF PROSTATIC ACID PHOSPHATASE

Choe, B., Pontes, E. J., Dong, M. K., Lillehoj, H. S., Wayne State University, School of Medicine, Dept. of Microbiology & Immunology, 1014 Rivard, Detroit, Michigan, 48207, United States of America

Acid phosphatase in human prostate (PAP) is antigenically unique secretory enzyme. Therefore, a few immunologic assay methods (RIA, EIA, IF) have been developed for this enzyme to explore the correlation between serum PAP levels or the PAP-secreting metastatic prostatic cancer cells in the biopsy materials with the clinical progression of prostatic carcinoma. In order to elucidate the molecular basis of antigenic specificity of PAP, monoclonal anti-PAP antibodies were prepared by hybridoma technique and these antibodies were tested against PAP, subunit polypeptides, and PAP-derived peptides. As a complementary approach, antibodies to acid phosphatase derived from the lysosomal fraction of human fibroblasts were prepared, and the antigenic difference between the PAP and other lysosomal acid phosphatases have been investigated.

BIBLIOGRAPHIC REFERENCES: E.J. Pontes, B.K. Choe, N.R. Rose: MA160 and EB33, HeLa cell contaminants or hybrids? In Vitro. 15:468-472, 1979. B.K. Choe, N.R. Rose, E.J. Pontes: Immunoenzyme assay for human prostatic acid phosphatase. Proceedings of the Society for Experimental Biology and Medicine, 162:396-400, 1979.

30. IMMUNOCYTOCHEMISTRY OF NORMAL AND NEOPLASTIC CELLS

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OBJECTIVE OF THE PROJECT: To investigate and search for specific markers for accurate identification of the various types of normal and neoplastic human cells.

RESEARCH PLAN AND METHODOLOGY: Several enzymes and tissue antigens with known cell or tissue specificities were investigated. This includes the acid phosphatase isoenzymes, the peroxidases, and the esterases. Standard cytotoxic and histologic techniques were used to prepare smears and sections for examinations. Ion exchange column chromatography, polyacrylamide gel electrophoresis, biochemical immunocytochemical enzyme assays involving the use of spectrophotometry, fluorometry and high pressure liquid chromatography were used.

FINDINGS, RESULTS OR CONCLUSIONS: A specific immunohistochemical method to detect prostatic acid phosphatase in tissues has been developed. This method was found to be highly specific and sensitive and is extremely useful in the diagnosis of metastatic carcinoma of the prostate. Cell markers for the tissue mast cells as well as for the osteoclasts have been identified and we are in the process of evaluating their clinical significance.

4. ISOENZYMES

31. THE USE OF ISOENZYME PROFILES OF LYSOSOMAL HYDROLASES IN DISTINGUISHING BETWEEN DIFFERENT TYPES OF LEUKEMIA

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OBJECTIVES: Ellis et al. (New Engl. J. Med. 1978, 298, 476-480) have recently shown that a raised activity of hexosaminidase isoenzyme 'T' is found in extracts of leukemic cells from children with the common form of childhood acute lymphoblastic leukemia but not in other forms of acute leukemia. This finding is to be assessed for its potential usefulness in the differential diagnosis of patients at presentation, in the early detection of relapse, and in prognosis. Also, the isoenzyme profiles of other lysosomal hydrolases will be studied in leukemic cells.

APPROACH: Isoenzyme profiles are determined using automated micro-column anion-exchange chromatography, and an attempt to prepare a specific hexosaminidase 'T' antiserum is being made.

PROGRESS: The investigation of a larger number of patients has confirmed the original findings. Pre-T ALL cells do not express hex 'T' and pre-B ALL cells have a lower activity than other ALL cells. Preliminary findings suggest that the acid phosphatase isoenzyme pattern is distinctive in T-ALL.

32. POPULATION VARIABILITY IN CARCINOMA OF THE BREAST

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The study aims to contrast the inherited characteristics of women with breast cancer with those of other groups and is drawing on two groups of patients. Blood samples are being analyzed at Durham University for a variety of red cell surface markers including the ABO, Rhesus, NMS, P, Kell, Duffy and Lewis blood groups. Red cell isoenzyme types, including acid phosphatase and esterase D, are being examined. The serum proteins, haptoglobin and transferrins are being studied. A number of patients are also being investigated for some HL-A types. Patients are collected from a combined breast lump clinic with free access, and blood samples are taken from all cases. The results will be analyzed in terms of differentiating benign and malignant breast lesions.

33. ISOENZYMES SPECIFIC TO HUMAN BREAST NEOPLASIA

Yang, N., Michigan Cancer Foundation, Dept. of Biology, 110 E. Warren Ave., Detroit, Michigan, 48201, United States of America

The limited evidence available suggests that most human cancers are likely the result of genetic transpositions. Through various rearrangements and accumulated mutations, which are now believed to occur commonly in human somatic cells, changes in activation and repression of specific genes, reflected in expression of enzymes, their isozymes and structural proteins, are considered to be the fundamental mechanisms by which the development and progression of a carcinoma are controlled. In this study, I intend to establish systematically the enzyme and isoenzyme differences and the differences in cell surface proteins between normal and malignant human mammary epithelial cells; the effect of mammotropic hormones on these differential expressions of isozymes and cell surface proteins; and whether this expression is useful in diagnosis and prognosis of human breast cancers.

The studies are currently being carried out using a defined system to culture normal and malignant mammary epithelial cells. Work in progress suggest that normal and malignant

human mammary epithelium exhibit differences in expression of certain specific enzymes and isozymes. Cell surface reactivities with specific plant lectins have also been found to distinguish normal from malignant breast tumor cells. Efforts are being made to characterize these cellular-biochemical properties unique to neoplastic human breast cells and to identify additional isozymes and cell surface proteins unique in type or amount in malignant human breast tumor cells. The possibility of using these characters as diagnostic and prognostic markers for breast cancer will be assessed.

34. PROPERTIES OF REGULATORY ENZYMES OF GLYCOLYSIS AND PURINE METABOLISM IN NEOPLASIA

Staal, G. E., Rijkse, G., van Veelen, C. W., Kraayenhagen, R. J., State University Hospital, Unit of Medical Enzymology, Catharijnesingel 101, 3511GV Utrecht, Netherlands

Pyruvate kinase isozymes were studied in normal brain tissue and in meningiomas and malignant gliomas. Pyruvate kinase from meningiomas and malignant gliomas is strongly inhibited by alanine, whereas pyruvate kinase from normal brain is not inhibited. Therefore the determination of the alanine inhibition of pyruvate kinase may be a diagnostic tool in surgery for gliomas (Thesis by C. van Veelen, in press). In view of the results obtained with the enzyme pyruvate kinase, two other enzymes will be studied in brain tumors: hexokinase and phosphofructokinase. The enzymes will be characterized with respect to isozyme shift, kinetics and electrophoresis. Furthermore, the possible correlation between malignancy and isozyme composition will be studied. The investigations will be carried out in collaboration with the Department of Neurosurgery of the Academic Hospital in Utrecht, The Netherlands.

Hexokinase was studied in cultured lymphoblasts (CEM-cell line). Besides hexokinase type I, type II is present too; normal lymphocytes have only type I. Furthermore, hexokinase was not influenced by inorganic phosphate. This means that some regulatory properties are absent. Methotrexate showed little or no influence on the hexokinase activity. Other regulatory enzymes such as phosphofructokinase, pyruvate kinase and some regulatory enzymes of purine metabolism will be studied with respect to isozyme composition and properties and the influence of methotrexate.

35. RADIOIMMUNOASSAY OF CREATINE KINASE ISOENZYMES

Zweig, M. H., McClean, S., Van Steirteghem, A., Feld, R., Silverman, L., Gazdar, A., Ihde, D., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, Clinical Center, Dept. of Clinical Pathology, 9000 Rockville Pike, Bethesda, Maryland, 20205, United States of America

Previously we developed a specific radioimmunoassay for the BB isoenzyme of creatine kinase (CK-BB). Currently, we are applying this assay to the measurement of CK-BB in human serum and other body fluids, human tumor homogenates, and cell cultures. Serum levels of CK-BB are an early and sensitive marker of acute myocardial infarction. Since serum levels of CK-BB are also elevated in the presence of a number of cancers, we have investigated the role of CK-BB as a tumor marker in patients with cancer of the lung, gastrointestinal and genitourinary tracts. It appears that CK-BB may be most useful in patients with small cell carcinoma of the lung.

BIBLIOGRAPHIC REFERENCES: Zweig, M.H., Adornato, B., Van Steirteghem, A.C. and Engel, W.K.; Serum creatine kinase BB and MM concentrations determined by radioimmunoassay in neuromuscular disorders. Ann. Neurol. 7, 324-328 (1980). Feld, R.D., Van Steirteghem, A.C., Zewig, M.H., Weimar, G.W., Narayana, A.S., and Witte, D.L.: The presence of creatine kinase BB isoenzyme in patients with prostatic cancer, Clin. Chim. Acta, 100, 267-273 (1980).

36. SERUM ISOENZYMES IN RELATION TO CANCER

Talawdekar, R. V., Srirat, A. V., Vyas, J. J., Rajpal, R. M., Vyas, J. J., Tata Memorial Hospital, Biochemistry Division, Dr. Ernest Borges Marg, Farel, Bombay 400012, Maharashtra, India

OBJECTIVE: The object of the current research project is to study whether the changes in the isoenzyme composition of the serum enzymes, which reflect the pattern of diseased organs, could have useful diagnostic application in cancer.

APPROACH: Biochemical.

PROGRESS: The work presently in progress is the study of isoenzyme composition of serum, salivary and urinary amylase in control subjects, lung cancer and salivary gland tumor. The project may take 2-3 years for completion. The work on isoenzyme studies of lactic dehydrogenase and aldolase in different types of cancer has been completed.

5. OTHER ENZYME MARKERS

37. DETERMINATION OF PROTEASES AND ANTIPROTEASES IN SERUM AND VAGINAL SECRETION OF PATIENTS WITH CERVICOUTERINE CANCER

Benitez Bribiesca, L., Freyre, R., Gallegos, G., Mendoza, M., Alfaro Aceves, L., Hospital de Oncologia, Division de Laboratorios, AV. Cuauhtemoc 330, Mexico City 7, D.F., Mexico

OBJECTIVE: To study the presence of proteases and antiproteases at various stages of cervicouterine cancer and determine their relationship to serum levels of the same proteins. In this fashion we will determine whether these proteases and antiproteases occur prior to the elevated serum levels associated with in situ and microinvasive cancer.

APPROACH: We will study 25 women in each of the following 6 groups: cervical carcinoma in situ, invasive carcinoma stages I, II, III, and IV, and acute cervical dysplasia. Two control groups will be comprised of women of comparable age without cancer or dysplasia and of women of comparable age with some form of cancer other than genital.

In all cases we will record age, menstrual cycles, use of hormonal contraceptives, presence of infection or other condition that may affect plasma levels of A-1-AT or proteases. Patients with cervical neoplasia will undergo exfoliative cytologic exam, biopsy, and in some cases conization. Controls will undergo only vaginal cytologic exam without biopsy.

Determinations will include: 1) cathepsin B, 2) trypsinic activity, 3) quantitative A-1-AT and alpha-2-macroglobulin, 4) total serum antiproteolytic activity, 5) total protein, and 6) electrophoresis in agar to observe alpha-1-globulin and A-2-macroglobulin bands.

Results will be tabulated and statistical analyses performed to compare all patient and control groups and obtain t and p values. In this fashion we hope to determine the stage of disease at which the substances under study appear.

38. DIAGNOSIS OF CANCER OF THE PANCREAS - INTERACTION BETWEEN PROTEASES AND PROTEASE INHIBITORS IN MALIGNANT DISEASE (ABBREV)

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The biochemical pattern of cancer of the pancreas: We aim primarily at developing and expanding our immunochemical methods for the analysis of proteins secreted by the pancreas in both juice and plasma. These methods will be tested in a clinical program dealing with cancer of the pancreas with the purpose of trying to evolve simple methods which can make possible an early diagnosis. Corresponding methods will be developed for the secretory proteins of rat pancreas. They will be evaluated in model studies of acinar cell injury and cancer induction.

A survey of the content of neutral proteases in cancer of the colon and the interaction with endogenous protease inhibitors: Preliminary results indicate the existence of collagenolytic activity in cancer of the colon. Our aim is to attempt to isolate the responsible enzyme in order to characterize and prepare an antiserum.

Studies dealing with the occurrence of the so-called bronchial inhibitor in normal plasma as well as in the case of inflammatory and neoplastic processes in the upper respiratory tract or in the genital tract: We have already produced a specific antiserum against the inhibitor and intend as an initial step to develop an RIA-method, which permits quantitation of the inhibitor at a level of 1 microgram/l.

Quantitative and qualitative studies of collagenase and elastase from neutrophilic granulocytes and their inhibitors in plasma in acute leukemia: Our specific antisera against proteases as well as inhibitors make an immunochemical approach possible. The results obtained shall provide a basis for future analyses

of possible harmful effects of these enzymes in cases of leukemia.

39. ENZYMATIC CYTOCHEMISTRY IN THE DIFFERENTIAL DIAGNOSIS OF RHABDOMYOSARCOMA

Santiago Payan, H., Benitez Bribiesca, L., Lerma, A., Hospital de Oncologia, AV. Cuauhtemoc 330, Mexico City 7, D.F., Mexico

OBJECTIVE: To differentiate and characterize the various types of rhabdomyosarcoma and tumors of round cells utilizing enzymatic cytochemical techniques commonly used in studying muscular lesions.

APPROACH: Cases of round cell neoplasia and various types of rhabdomyosarcoma will be selected by transoperative study or from known recurrences. Fragments of fresh tissue will be obtained and divided into three groups. Samples in the first group will be fixed in formaldehyde, encased in paraffin and stained; in the second - processed and encased in plastic, cut by ultramicrotome, stained and studied under the electron microscope; in the third - frozen in N2 isopentane liquid and cut to 4 microns for enzyme histochemistry.

The enzymatic techniques will be: Ca-dependent muscular ATPase, succinodehydrogenase, lactodehydrogenase, NADH reductase, acid phosphatase, alkaline phosphatase and myophosphorylase.

Observations will be made using an optical microscope, and comparing results obtained using routine histologic techniques with those obtained when the cytoenzymatic techniques were employed. In turn, these results will be compared with those obtained by electron microscope and an attempt will be made to identify the cellular types and evaluate if the cytoenzymatic techniques help for differential diagnosis.

40. BIOCHEMICAL AND IMMUNOLOGICAL MARKERS OF DIAGNOSTIC AND PROGNOSTIC IMPORTANCE IN ORAL CANCER

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About 40% of the cancer cases detected and treated in Gosalia Memorial Hospital run by the Goa Cancer Society, are cancers of buccal cavity, pharynx and larynx. Leukocyte adherence inhibition assay was shown to be useful in detection of cancer. An extensive study is planned to search for biochemical and immunological markers of diagnostic and prognostic importance in oral cancer. Enzymes such as amylase, G-6-phosphate dehydrogenase, lactic dehydrogenase, phosphohexose isomerase, alkaline phosphatase and their isoenzymes, ectopic hormones such as human chorionic gonadotropin and human placental lactogen and immunological marker carcinoembryonic antigen will be measured in saliva, serum and urine of oral cancer patients. In addition, leukocyte adherence inhibition assay will be done before and after treatment.

BIBLIOGRAPHIC REFERENCES: Use of leukocyte adherence inhibition test to study the cell mediated antitumor immunity in cancer patients. S.R. Shirodkar and S.G. Gangal. Ind. J. Med. Res. 70, 88-96 (1979).

41. TUMOR ENZYMES AND PROGNOSIS IN HUMAN BREAST CANCER

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As explained in last year's report (ICRF Scientific Report 1978 -report 35), this clinical project on the investigation of biochemical parameters as aids to prognosis is continuing. To date, we have received tumors from about 600 patients and we are awaiting recurrences amongst these cases. The possibility of using enzymes of carbohydrate metabolism as possible indicators of future recurrence has been detailed in previous reports and awaits confirmation.

42. INDUCIBILITY OF ARYL HYDROCARBON HYDROXYLASE IN CULTURED LYMPHOCYTES FROM LUNG CANCER PATIENTS AND CONTROLS

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The overall objective of this study is to assess the potential usefulness of the lymphocyte aryl hydroxylase test as a tool for epidemiological studies on genetic susceptibility to chemical carcinogens. The activity and inducibility of AHH in lymphocytes vary widely between subjects. Kellermann and co-workers (1973) suggested a higher susceptibility to lung cancer, caused by carcinogens in cigarette smoke, in subjects with higher inducibility of this enzyme. Since the inducibility of AHH is genetically controlled, some subjects appeared to be genetically predisposed to this type of cancer. However, the test determining the AHH inducibility in lymphocytes was never applied in preventive medicine; there has been criticism of the reproducibility of the method and the implications of the results. Nevertheless, as several recent papers supported a relationship between agents, patients with lung and other types of cancer and healthy volunteers are being studied in respect of the distribution of AHH inducibility. The method is being improved to make it more suitable for testing cancer patients.

43. ENZYMES OF NORMAL AND MALIGNANT INTESTINAL MUCOSA

Balls, M. E., Trotta, P. P., Yip, L. C., Relyea, N. M., Sloan Kettering Inst. for Cancer Research, 410 E. 68th St., New York, New York, 10021, United States of America

To examine a number of slides of patients from the mid 1970's whose subsequent course is known to see if our TK antibodies reveal abnormalities consistent with the known history. To assay fresh surgical specimens using TK and PRPP synthetase antibodies and correlate these results with other laboratory and clinical data. To attempt to prepare monoclonal antibodies that may enhance the specificity and sensitivity of these antibodies. We shall attempt to purify the factor in tumors that enhances the action of DCF and the inhibitor of ODC in colonic mucosa.

BIBLIOGRAPHIC REFERENCES: P.P. Trotta, Mary P. Ahland, G.F. Brown and M.E. Balls. Studies on the Effects of Infusion of Enzyme Inhibitors on Mouse Adenosine Deaminase. *Molecular Pharmacology*, 14, 91-101, 1978. P.P. Trotta and M.E. Balls. Characterization of Adenosine Deaminase from Normal Colon and Colon Tumors. Evidence for Tumor-Specific Variants. *Biochemistry*, 17, 270, 1978.

44. RADIOIMMUNOASSAY OF TYROSINASE AND MELANOMA DETECTION

Gochman, N., Baird, S. M., U.S. Veterans Admin., Dept. of Medicine & Surgery, Medical Center, Clinical Chemistry Section, 3350 La Jolla Village Blvd., San Diego, California, 92161, United States of America

We propose to develop a specific radioimmunoassay (RIA) for tyrosinase in human serum that could be used for the early detection, laboratory diagnosis and serial monitoring of malignant melanoma. The tyrosinase RIA would provide the sensitivity and specificity that is lacking in current laboratory diagnostic methods.

The proposed program will have the following objectives: (1) Purification of tyrosinase from human melanoma tissue. (2) Preparation of antiserum against the purified tyrosinase. (3) Radioiodination of the tyrosinase to provide a labeled antigen for the RIA. (4) Development of the assay conditions for the RIA, including methods of separation of antibody-bound and free tyrosinase. (5) Validation of the RIA for cross-reactivity, sensitivity, precision and other analytical parameters. (6) Clinical evaluation of the RIA by testing of serum samples from human subjects who are normal, who have non-melanoma tumors, and who have various stages of melanoma.

Current experiments have involved the generation of specific antibodies in rabbits to tyrosinase obtained from various sources, including mushrooms and murine melanoma tissue. Efforts are underway to obtain sufficient melanoma tissue from human sources so that tyrosinase can be obtained for purification and antibody production. Chromatographic isolation procedures for tyrosinase have been established using murine melanoma tissue, that will serve as a model for human tissue experiments. Simple radio-iodination of purified tyrosinase has proven to be difficult, and additional approaches using derivatization will be attempted.

45. HISTAMINASE AS A BIOCHEMICAL MARKER FOR HUMAN CANCER

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Previous research results from our own and other laboratories have established an association of increased histaminase levels with a number of human cancers. Based on this association and the fact that the enzyme is produced at high level in placenta but at very low level in most normal tissues, research efforts have been made in our laboratory to establish histaminase as a onco-placental enzyme. This is carried out by the comparison of the biochemical and immunological characteristics of this enzyme from the placenta and from malignant effusion fluids and tumors. A monospecific anti-serum against placental histaminase reacts with the enzyme in tumors and in malignant effusion fluids. Using this antiserum, immunohistochemical staining methods for this enzyme have been developed.

We now propose to further evaluate the potential of histaminase as a biochemical marker for human cancer. This will be done by first developing a sensitive radioimmunoassay for histaminase. Using this method, together with the immunohistochemical methods, we will study this enzyme in sera, malignant effusion fluids, tumor cells and tumor tissues of cancers of various types at various stages of the disease. Results will be evaluated for the correlation of the presence of this enzyme with the type and the stage of the cancer and with the histopathological characteristics of the tumor. Biochemical and immunological studies of this enzyme in placenta and in tumor will be carried out to obtain basic information pertinent to the understanding of the significance of this enzyme in normal and in tumor cells.

This study, if successfully accomplished, will be a major step toward the development of a test using histaminase for the detection and monitoring of the cancer.

46. ENZYMES AND CANCER

Hutton, J. J., U.S. Veterans Admin., Dept. of Medicine & Surgery, Medical Center, Hematology Section, San Antonio, Texas, 78284, United States of America

This investigator transferred from VAMC, Lexington, Ky., project number 004.

The objectives of this project are (a) to characterize enzymes playing unique roles in DNA metabolism of human lymphocytes and (b) to develop new strategies of therapy of lymphoma-leukemia by taking advantage of properties of these enzymes. Our initial plan was to purify and characterize 3 relevant enzymes from human cells. Those selected were adenosine deaminase, terminal transferase, and purine nucleoside phosphorylase. Leukemia cells were used as starting material and affinity chromatography was the major technique of purification. We have purified each enzyme to homogeneity and have characterized them extensively. We have prepared antisera to each protein and have described a radioimmunoassay of adenosine deaminase and an immunocytochemical procedure for detecting transferase in cells. Both terminal transferase and adenosine deaminase serve as useful markers of cell type in lymphoid leukemia and lymphomas. At the present time we are testing inhibitors of these enzymes as agents for the treatment of human cancer. Most of the measurements have been carried out in vitro except for clinical trials of deoxycytosine, which kills lymphoid cells in cell culture. Deoxycytosine inhibits adenosine deaminase and is being tested clinically for activity against lymphoid malignancies.

BIBLIOGRAPHIC REFERENCES: J.J. Hutton, M.S. Coleman, R.P. Keneklis and F.J. Bollum. Terminal Deoxynucleotidyl Transferase as a Tumor Cell Marker in Leukemia and Lymphoma, Results from 1000 Patients: in *Biological Basis for Cancer diagnosis*. M. Fox, ed. Pergamon Press Ltd., Oxford, 1979.

47. ESTERASE ACTIVITY IN HODGKIN'S DISEASE PATIENTS

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This is part of a broader project. A summary of this subproject is not available.

48. COLLAGENASE AND INVASION OF HEAD AND NECK TUMORS

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The objective of this project is to analyze and characterize a collagenolytic enzyme from a variety of head and neck tumors and to determine the significance of collagenolysis in the invasiveness and clinical behavior of these malignancies in humans and in an experimental epidermoid tumor in rats.

The current research was to compare the levels of collagenase and protease activities according to the anatomic site, histological pattern (i.e., squamous versus nonsquamous), tumor size and tumor differentiation. We have also studied the effect of prostaglandin (possibly a chemical mediator for tumor growth and spread) on the synthesis and the release of collagenase and protease. The animal model also will be used to study immunoinhibition of collagenase in vitro and in vivo to test whether immunoinhibition of collagenase will decrease tumor invasiveness and improve animal survival.

We hope that as a result of these studies, a treatment plan for head and neck tumors can be placed on a more rational basis.

BIBLIOGRAPHIC REFERENCES: Huang, C.C., Wu, C.H., and Abramson, M.: Collagenase Activity in Cultures of Rat Prostate Carcinoma. *Bioch. Biophys. Acta* 570:149-156, 1979.

49. EXTRACELLULAR ROLE OF THIOL PROTEINASES IN INVASIVE TUMOR GROWTH AND METASTASIS

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OBJECTIVE: Investigation of the role of proteinases, particularly thiol proteinases, in tumor growth and metastasis.

APPROACH: The secretion of a thiol proteinase from malignant human and mouse mammary carcinomas has been observed in organ culture. Factors controlling the secretion of this enzyme are being investigated. Purification of the enzyme is being undertaken to allow biochemical characterization and to study its effect on cell surfaces and components of the intercellular matrix. The purified enzyme will also be used to produce a monospecific antiserum using either conventional or monoclonal antibody techniques. Immunolocalization studies will then be used to characterize its cellular origin.

PROGRESS: A comparative study of the secretion of proteinases from human mammary tumors maintained in organ culture revealed that malignant tumors secreted increased levels of a cathepsin B-like thiol proteinase when compared to fibroadenomas or normal tissue. In comparison the secretion of other proteinases - cathepsin D, collagenase and plasminogen activator - showed no significant difference between malignant and benign tumors.

A thiol proteinase similar to that secreted by malignant human tumor was also found to be secreted by spontaneous mammary tumors in C3H/j (retired breeder) mice. The enzyme from both species has enzymatic properties similar to those of cathepsin B but not unlike this lysosomal proteinase it is stable at neutral pH and has physical properties different from cathepsin B. This thiol proteinase is actively secreted from tumor tissues as its release is strictly dependent on protein synthesis. Hormone studies indicate that secretion is not affected by the presence of insulin or estradiol in the culture medium. Furthermore, there was no correlation in human tumor specimens between the capacity to secrete thiol proteinase and estrogen receptor content.

50. ENDOGENOUS PEROXIDASE - MARKER PROTEIN IN ESTROGEN-DEPENDENT TUMORS (HUMAN, MICE)

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This is part of a broader project. A summary of this subproject is not available.

51. LYMPHOMA - DIAGNOSIS BY BLOOD AND LIVER TESTS

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Preliminary comparisons of the activities of several enzymes in liver and blood components of control and cancer patients have indicated substantial quantitative changes in the activities of enzymes in liver (arginase, peptidyl proline hydroxylase, alanine amino-transferase, hexokinase, glucose-6-phosphate dehydrogenase, thymidine kinase (particulate), pyrroline-5-carboxylate reductase (cold Stable), and glutamate dehydrogenase (soluble)) and in blood plasma (gamma-glutamyl transferase, arginase), erythrocytes gamma-glutamyl transferase, arginase, pyrroline-5-carboxylate reductase), and leukocytes (gamma-glutamyl transferase, arginase) in cases of clinically diagnosed cancer. Abnormally high activities of gamma-glutamyl transferase in liver and blood appear to be associated with cancer of the pancreas while raised arginase and plasma glutamate dehydrogenase levels may distinguish patients with hepatic malignancies from all others. Although many more analyses on a wider spectrum of controls and cancer patients will be required before the significance of these preliminary observations can be assessed, the tentative results suggest that tumor marker enzymes may be identifiable in human blood and quantitative determinations of their activities may eventually contribute a non-invasive technique for screening and monitoring patients.

Extensions of preliminary studies of enzyme activities of human tissues (fetal and adult) have confirmed our hypothesis that the enzyme composition of host tissues (liver and lung) represents an intermediate stage between fetal and adult congener, reflecting more fetal characteristics than the normal adult and fewer than tumors themselves. Quantitative enzyme abnormalities in morphologically normal host tissues (liver and lung not involved with neoplastic growth) may be detected easily by available methods and may offer a refinement in the diagnosis of and prognosis for cancer patients.

BIBLIOGRAPHIC REFERENCES: Herzfeld, A., Greengard, O., and McDermott W.V.: Enzyme pathology of the liver in patients with and without non-hepatic cancer. *Cancer*, in press, (1980). Herzfeld, A. and Greengard, O.: Enzyme activities in human fetal and neoplastic tissues. *Cancer*, in press, (1980).

52. STUDIES ON BIOCHEMICAL MARKERS IN HUMAN BREAST CANCER, LEUKEMIA AND LYMPHOMA

Skoog, L., Karolinska Sjukhuset, Dept. of Tumor Pathology, S10401 Stockholm 60, Sweden

OBJECTIVE: To evaluate the use of biochemical markers in subdividing human tumors with respect to malignancy.

APPROACH: Four enzyme markers (TdT, adenosinedeaminase, 5' nucleotidase and hexosaminidase) and cytosol glucocorticoid receptor levels are measured in the lymphoma-leukemia group. The breast cancer studies include determination of estrogen receptor levels and plasma membrane composition.

53. MOLECULAR PATHOLOGY OF LEUKEMIA AND LYMPHOMA

Coleman, M. S., Riley, L., Cibull, M., Jacob, W., Danton, M. J., University of Kentucky, School of Medicine, Dept. of Biochemistry, Limestone & Euclid, Lexington, Kentucky, 40506, United States of America

We and others have found that there are unique DNA synthesizing enzymes in precursors of lymphoid cells that may serve as tumor markers. A group of enzymes (TdT, ADA and PNP) have been selected as potential markers of lymphoid precursors. We have emphasized the development of immunological tools to detect these enzymes in small quantities of cells. It is our opinion that these techniques will ultimately be extraordinarily useful in clinical management of patients with leukemia and lymphoma.

BIBLIOGRAPHIC REFERENCES: Wiginton, D.A., Coleman, M.S., and Hutton, J.J. Metabolism of Deoxyribonucleosides and Ribonucleosides by Purine Nucleoside Phosphorylase Purified from Human Leukemic Granulocytes. *Journal of Biological Chemistry*, 257, 6662-6669, (1982). Siaw, M.F.-E., Mitchell, B., Koller, C., Coleman, M.S. and Hutton, J.J., Biochemical Consequences of Deoxycytosine

B. OTHER PROTEIN AND PEPTIDE MARKERS IN CANCER DIAGNOSIS AND PROGNOSIS

54. POPULATION VARIABILITY IN CARCINOMA OF THE BREAST

Cartwright, R. A., Cartwright, S. C., Sunderland, E., Yorkshire Regional Cancer Org., Leeds LS16 6QB, England, United Kingdom

The summary for this project is under entry no. 32.

55. SIGNIFICANCE OF CELLULAR RETINOIC ACID BINDING PROTEIN, ORNITHINE DECARBOXYLASE AND POLYAMINES AS POSSIBLE MARKERS IN PRENEOPLASIA

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OBJECTIVE: Retinol (vitamin A), retinoic acid and their synthetic analogs, commonly known as retinoids, are highly promising agents which are able to prevent or induce reversal of cell transformation. At this time, the mechanism of action by which retinoids exert their effect is not yet known. It has been proposed that retinoids behave like steroids, since specific cellular binding proteins for retinoic acid (cRABP) were found in several developing, as well as fast dividing and tumorous, tissues. cRABP was, therefore, suggested to be used as a biochemical marker for early detection of cancer.

APPROACH: During the last two years significant progress was made in determining, by various methods, the specific binding protein for retinoic acid. This method allows quantitative determination of the specific cellular binding protein for retinoic acid and is very accurate and highly sensitive.

PROGRESS: Results from our laboratory show that specific binding for retinoic acids occurs not only in cancerous tissue of human mammary tumors, but also in preneoplastic stages (simple and proliferative dysplasia). Only very little activity of ornithine decarboxylase could be found in human mammary tissue which does not allow use of this enzyme as a biological tumor marker.

56. AN IMMUNODIAGNOSTIC ASSAY FOR BREAST CANCER DETECTION

Gaffney, E. V., Pennsylvania State University, University Park Campus, School of Science, Dept. of Microbiology Cell Biology Biochemistry & Biophysics, 211 Whitmore Lab., University Park, Pennsylvania, 16802, United States of America

The current proposal concerns the development of a diagnostic approach to breast cancer which will correlate the level of a growth inhibitory factor in sera with the presence or absence of breast carcinoma. Initial studies show that human serum (HuS) from normal donors specifically inhibits the growth of malignant mammary cell populations. This same activity is absent or diminished in sera from breast cancer patients. The activity is attributable to a protein component which is partially purified. This study takes advantage of the sensitivity of the MCF-7 cell line to examine sera samples from different population groups and thus determine what effect variations of sex, age and health status have on the level of activity. In addition, isolation and characterization of this factor will be pursued with a view to the eventual development of a diagnostic radioimmunoassay. The tissue specificity of this activity will be studied by determining the growth response to HuS of malignant cells derived from other cancer types.

57. BIOLOGIC MARKERS IN BREAST CANCER PATIENTS

Sussman, H. H., Stanford University, School of Medicine, Dept. of Pathology, 300 Pasteur Dr., Stanford, California, 94305, United States of America

OBJECTIVES: Conduct a longitudinal study of biologic markers in breast cancer patients. Evaluate tissue and serum markers currently proposed as being potentially useful and will develop a new set of markers from placental membrane proteins and glycoproteins and investigate their presence in serum and

tissue. These studies will be coordinated with a well defined clinical study of breast cancer sponsored by the Northern California Cancer Program and conducted by the Northern California Oncology Group. This will provide a set of patients whose clinical care will be rigorously controlled and will also be to ensure direct control of specimens and accurate patient information. There will be twelve major institutions participating in these studies. The NCOG protocols will evaluate the patients by routine tests, and these will be complemented by our evaluation of the markers that will be measured in this protocol.

58. PURIFICATION OF HUMAN BREAST TUMOR PROTEINS

Lerner, M. P., Nordquist, R. E., University of Oklahoma, Health Sciences Center, School of Medicine, Dept. of Microbiology & Immunology, Box 26901, Oklahoma City, Oklahoma, 73190, United States of America

Breast cancer is a major cause of death of American women. However, there is little information on biochemical and immunological events that occur in human breast tissue during the neoplastic process. Our past studies have shown that sera from women with breast cancer have antibodies reactive with a human breast tumor cell line, BOT-2 cells. Our recent studies show that the proteins isolated from BOT-2 cells are reactive with sera from breast cancer patients. Preliminary studies indicate that the proteins isolated from BOT-2 cells are quite similar, biochemically, to proteins isolated by others from whole human breast tumor tissue. The objectives of this proposal are to isolate and purify the proteins from BOT-2 cells using standard chromatographic and electrophoretic methods. The data will indicate the biochemical nature of human breast tumor proteins and may be quite useful in future serodiagnostic, therapeutic, and immunologic studies of human breast cancer.

BIBLIOGRAPHIC REFERENCES: Schafer, F.B., M.P. Lerner, J.H. Anglin, and R.E. Nordquist. 1979. Is shedding of Immune System Inhibitors responsible for the survival of human melanoma tumors? *Pigment Cell* 5:169-173.

59. ISOPROTEINS IN NORMAL AND MALIGNANT BREAST TISSUES

Balinsky, D., Iowa State University of Science & Technology, College of Sciences & Humanities, Dept. of Biochemistry & Biophysics, Beardshear Hall, Ames, Iowa, 50010, United States of America

OBJECTIVE: To detect, quantitate and characterize various isoenzymes or non-enzymic isoproteins in normal, benign and neoplastic breast tissue. These findings might help in the detection of breast cancer at an early stage, or of disseminated breast cancer following local treatment of the primary cancer.

60. PANCREATIC SECRETORY PROTEINS IN CANCER OF THE PANCREAS

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The aim of the proposed work is to analyze pancreatic secretions of patients suspected of having cancer of the pancreas or with a positive diagnosis of cancer of the pancreas and to compare them with secretory profiles obtained from healthy volunteers in the hope of detecting qualitative or quantitative abnormalities that might help in the early diagnosis of this disease. These studies will be accompanied by a similar investigation in hamsters during induction of pancreatic neoplasms with nitrosamines. Pancreatic tissue and fluid of groups of treated and untreated animals will be analyzed at regular intervals for changes in various parameters which in human patients have indicated the presence of pancreatic disease. Thus, ratios of trypsinogen variants, trypsin inhibitor, chymotrypsinogen and a number of lysosomal enzymes will be determined. Highly sensitive methods developed previously for investigation of human pancreatic fluid will be adapted for pancreatic tissue and fluid from hamsters. Data obtained from these assays will be correlated with concurrent histological studies in order to explore the possibility that changes in secretory proteins may precede recognizable histological abnormalities characteristic of tumor cells. Such changes or combination of changes might serve as sensitive markers for present or incipient pancreatic pathology.

BIBLIOGRAPHIC REFERENCES: Trypsinogen Variants in Pancreatic Juice of Healthy Volunteers, Chronic Alco-

holics and Patients with Pancreatitis and Cancer of the Pancreas. H. Rinderknecht, I.G. Renner and C. Carmack. *Gut* 20:886-891 (1979). Trypsinogen Variants in Pancreatic Juice of Healthy Volunteers, Chronic Alcoholics and Patients with Pancreatitis and Cancer of the Pancreas. H. Rinderknecht, I.G. Renner and C. Carmack. *Medical Digest (Gastroenterology Digest)* in press (1980).

61. EARLY DIAGNOSIS OF PANCREATIC CANCER

Geokas, M. C., DelMar, E. G., Largman, C., Brodrick, J. W., University of California, Davis Campus, School of Medicine, Dept. of Internal Medicine, Davis, California, 95616, United States of America

The main goal of this research is to determine whether a protein(s) specific for pancreatic carcinoma is present in serum samples obtained from patients with this disease. Previous attempts to detect such a protein in this laboratory by immunological techniques, including production of cancer specific antisera through the use of fetal pancreas extract of pancreatic cancer tissue, have been unsuccessful. We have therefore turned our attention to the detection of a protein specific for pancreatic cancer in patient sera by the two-dimensional gel electrophoresis procedure developed by O'Farrell (*J. Biol. Chem.* 250, 4007, 1975). Several methods for enhancing the sensitivity and resolving power of this technique for the detection of minor proteins in serum or plasma are currently being evaluated, including selective removal of albumin, a sensitive silver staining procedure, and detection of proteins by autoradiography after labeling by reductive methylation of lysine residues with 14C-formaldehyde. It is expected that the optimized method will be capable of detecting protein spots at an abundance of 10 to the minus 2nd power to 10 to the minus 3rd power percent of total protein. O'Farrell has previously demonstrated that two-dimensional gel electrophoresis can resolve 1100 distinct protein spots in a bacterial cell extract. The technique will be applied to the screening of sera from patients with pancreatic cancer and normal controls in an attempt to detect qualitative changes in the protein pattern that are specific for pancreatic cancer.

The need for early diagnosis of carcinoma of the pancreas is great and we believe that the accomplishment of the goals of this research might eventually lead to a significant breakthrough in improving resectability and survival rate.

62. PANCREATIC DUCTAL VERSUS DUODENAL SECRETIONS

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The authors are attempting to find tumor markers in pancreatic secretions obtained from human beings at surgery and by ERCP, then to separate the proteins by isoelectric focusing and, finally, analyze the protein patterns using a densitometer which has been coupled with a small computer. The computer then averages and determines statistically the gravities of proteins in each peak, and compares normal secretions from those of patients with cancer and chronic pancreatitis.

BIBLIOGRAPHIC REFERENCES: White, T.T., Slavotinek, A.H.: Results of surgical treatment of chronic pancreatitis. Report of 142 cases. *Ann. Surg.* 189:217-224, 1979. Tatarchuk, T.W., White, T.T.: A new instrument for inserting a U-tube. *Am. J. Surg.* 137:425-426, 1979.

63. LONGITUDINAL STUDIES OF A POTENTIAL TUMOR MARKER IN THE SERUM OF PATIENTS WITH MALIGNANT LYMPHOMA

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Using a method developed primarily for the detection and characterization of circulating immune complexes in malignancy, analysis of material precipitating at low concentrations of polyethylene glycol by SDS-PAGE showed the presence of an extra protein band associated with lymphomas (ICRF Scientific Report 1978 - report 239). The serum factor had an apparent molecular weight of 23,000 daltons and is provisionally referred to as T23.

Preliminary data indicating that the appearance of T23 was disease-related have been followed by a more extensive investi-

gation of the incidence of T23 in serial samples from 25 patients with Hodgkin's disease and 33 with non-Hodgkin's lymphoma.

T23 was detected in sequential samples from nine out of twelve patients with active Hodgkin's disease whereas its incidence was greatly reduced during response to chemotherapy (three out of thirteen) and in remission (three out of eleven). Of the three patients who had detectable T23 during remission, one relapsed within one month of the positive result, although the second has been clinically free of disease for one year. The third patient became positive three weeks after discontinuing chemotherapy but did not relapse in the ensuing two months.

A similar result was obtained from studies on non-Hodgkin's lymphoma with T23 present in the majority of patients with active disease (fifteen out of twenty-four), to a lesser extent during successful chemotherapy (six out of nineteen) and undetectable in any of seven cases in complete remission.

The correlation between active disease and presence of T23 indicates that measurement of this serum factor may be of use in the management of lymphomas. Investigation of its clinical potential is continuing.

64. PROTEINS OF LYMPHOCYTES AND MACROPHAGES

Shifrin, S., Parrott, C. L., Merrill, C. R., Lipkin, L., Ebert, M., Klee, W., Mizel, S., Gottesman, M., Switzer, R., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Macromolecular Biology Section, 9000 Rockville Pike, Bethesda, Maryland, 20205, United States of America

The purpose of this project is to examine the proteins and peptides of lymphocytes, cerebrospinal fluid, urine and plasma obtained from a family with Alzheimer disease, using two dimensional gel electrophoresis. Lymphocytes were examined directly after separation on Ficol/Hypaque or stimulated with PHA to incorporate 35S-methionine. Other clinical samples were analyzed directly on two dimensional gels using a super-sensitive silver stain developed during the course of this study.

BIBLIOGRAPHIC REFERENCES: Switzer III, R. C., Merrill, C. R., and Shifrin, S.: A highly sensitive silver stain for detecting proteins and peptides in polyacrylamide gels. *Anal. Biochem.* 98: 231-237, 1979.

65. IDENTIFICATION OF TUMOR RELATED GLYCOPROTEIN TUMOR MARKERS IN MALIGNANT MELANOMA

Silver, H. K., Cancer Control Agency of British Columbia, Dept. of Advanced Therapeutics, 2656 Heather St., Vancouver, British Columbia, V5Z 3J3, Canada

OBJECTIVE: To identify tumor related glycoproteins in serum and evaluate their use as clinical tumor markers.

APPROACH: Tumor related glycoproteins have been identified in malignant and transformed cells, in spent tissue culture medium used to grow neoplastic cells, and in the serum of cancer patients. These can be most readily monitored by measurement of glycoprotein bound sialic acid. Initial studies have demonstrated close correlation with tumor burden in malignant melanoma, breast carcinoma, and ovarian carcinoma. Investigation is in progress to evaluate the clinical value of these measurements in other malignancies. Further preliminary studies have suggested that the non-specific acute phase reactant glycoproteins can be quite readily separated from the more specific glycoproteins of tumor origin.

66. THE EVALUATION OF MELANOMA-SPECIFIC PROTEIN AS A TUMOR MARKER

Hobbs, J. R., Cooke, K. B., University of London, Westminster Medical School, Dept. of Chemical Pathology, 17 Horseferry Rd., London SW1P 2AR, England, United Kingdom

OBJECTIVE: To maintain the fluorescence-inhibition assay currently used to measure melanoma-specific protein (MSP) in the blood and urine of patients with benign and malignant pigment diseases. To survey its value as a monitor of pigment cell diseases, including malignant melanoma. To try to improve on the assay by producing either a radioimmunoassay or enzyme-linked assay, hitherto not possible due to the apparent single antigenic site present on MSP. To also assess if MSP emerges when normal pigment cells are exposed to toxic materials, irradiation or PUVA.

APPROACH: Standard anti-MSP antiserum has hitherto been raised in rabbits immunized with a fraction isolated from the urine of patients with malignant melanoma. We will now attempt to raise hybridoma antibodies in mice or rats against this particular fraction. The antisera produced will be compared with our existing anti-MSP, for, as is becoming apparent with monoclonal antibodies, there is a whole range of anti-melanoma antibodies resulting from the use of human melanoma tissue cultures. Some of these cross-react, but we are particularly anxious to retain the anti-MSP activity, which has proved so valuable in the past.

PROGRESS: MSP in urine has been found in 91% of patients with malignant melanoma of stage 3 and worse, and in 76% of patients with an apparent primary malignant melanoma. It is only detectable in some 50% of patients in intermediate status. It rises with relapse. Among other diseases it has only been found with halo naevus or indeed with very active vitiligo of rare varieties. It has not been found in a vast array of skin diseases with pigment disorders.

67. STUDIES OF HUMAN PROSTATIC CANCER CELLS IN VITRO

Mariyama, K., Mochizuki, S., Miyauchi, M., Kawamura, K., Fukushima, T., Nagayama, T., Chiba Cancer Center Research Inst., Dept. of Pathology, 666-2, Nitona-Cho, Chiba 280, Chiba, Japan

OBJECTIVE: Continuous propagation of human prostatic cancer (PCa) cells in vitro as a possible source of specific marker proteins useful in diagnosis of human PCa.

APPROACH: Characterize cells grown in vitro from human PCa in terms of biological (growth behavior in culture and in nude mice), morphological (light and electron microscopy), karyological (specific bandings), antigenic (human prostatic acid phosphatase antigen), and other parameters. Test hormones and other chemicals for possible promoting effects on growth of cultured human PCa cells.

PROGRESS: A number of cultures identified as highly suggestive of PCa cell origin have been obtained from biopsies of human PCa tissues and maintained for over 30 passages in vitro. Different substances are currently being tested for possible growth promoting effects.

68. EVALUATION OF HUMAN EMBRYONIC PREALBUMIN AS A TUMOR MARKER

Horne, C. H., University of Aberdeen, Dept. of Pathology, Aberdeen AB9 1AS, Scotland, United Kingdom

OBJECTIVE: The aim of this study is to evaluate the role of the recently identified fetal plasma protein, human embryonic prealbumin (EPA) as a marker for the detection and monitoring of patients with malignant connective tissue tumors.

APPROACH: Following the isolation and purification of EPA a sensitive radioimmunoassay for the measurement of EPA in serum and tissue homogenates will be developed.

By screening selected pre- and postoperative sera from patients with malignant mesodermal tumors for EPA it will be possible to determine the usefulness of EPA as a marker for the early detection of tumor recurrence.

69. RETINOL BINDING PROTEIN IN CANCER PATIENTS

Haines, A. P., Thompson, S. G., Bass, T. K., Hunt, R., Medical Research Council, Dept. of Health & Social Security, Epidemiology & Medical Care Unit, Watford Rd., c/o Northwick Park Hospital, Harrow HA1 3UJ, England, United Kingdom

Previous studies have shown that patients with lung cancer have lower serum vitamin A levels than comparison patients with non-malignant disease. These differences may be due to or may antedate the tumor. In order to determine whether vitamin A levels were lower in patients who develop cancer before the tumor had become clinically manifest, stored plasma samples from patients in a prospective study of heart disease (the Northwick Park Heart Study) who went on to develop a tumor after the blood sample has been taken, have been analyzed. Each index case has been compared with three comparison cases who did not develop a tumor over the follow-up period. The average period between the blood sample and diagnosis of cancer was about three years. Unfortunately measurement of vitamin A itself was impossible because of an artefact produced on storage of the plasma which interferes with spectrophotometry. Retinol binding protein has therefore been measured immunologically.

A total of 26 cases of cancer with comparison cases have been analyzed.

70. SIALOGLYCOPROTEINS IN THE CLINICAL MANAGEMENT OF HUMAN NEOPLASIA

Silver, H. K., Cancer Control Agency of British Columbia, Dept. of Advanced Therapeutics, 2656 Heather St., Vancouver, British Columbia, V5Z 3J3, Canada

OBJECTIVES: a) To identify and characterize human tumor-associated sialoglycoproteins in cancer patients' sera and tumor cell culture. b) To evaluate the clinical significance of sialoglycoproteins as monitors of tumor burden.

APPROACH: We have demonstrated that measurement of serum sialoglycoproteins, as reflected by total serum sialic acid (N-acetyl neuraminic acid), is a tumor marker applicable to a broad spectrum of tumor types. We have also found that tumor-associated sialoglycoprotein can be partially separated from acute-phase reactant sialoglycoprotein by relatively simple biochemical fractionation. We will use this as a basis for a refined sialic acid assay and compare our investigations with previous clinical studies. We will concurrently evaluate individual sialoglycoproteins by higher resolution fractionation procedures and polyacrylamide gel electrophoresis.

71. ECTOPIC PROTEIN AND SUBUNIT PRODUCTION BY TUMORS

Rosen, S. W., Weintraub, B. D., Morrow, J. S., Aaronson, S., Osborne, J., Heyderman, E., Torney, D., Horne, C., Zopf, D., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Inst. of Arthritis Metabolism & Digestive Diseases, Endocrine Physiology Section, 9000 Rockville Pike, Bethesda, Maryland, 20205, United States of America

We continue to study placental proteins as cancer markers. The pregnancy-specific beta1 glycoprotein (SP1) is produced by normal human fibroblasts but does not appear to be a useful marker in cases of fibrosarcoma, osteogenic sarcoma, glioma or breast carcinoma. Placental protein 5 is also made by human fibroblasts in vitro but its regulation by the cell appears to be different from that of SP1. Chemical modification of SP1 is being explored in an attempt to understand its unusual CD spectrum. Placental lactogen (PL) is made by different cells of a testicular choriocarcinoma than is alpha subunit. Large forms of PL have been detected in serum and placenta at term, even when blood and tissue have been rapidly processed. Analysis of oligosaccharides released from CG-alpha by trifluoroacetylation has revealed heterogeneity unrecognized in current structural formulas of this glycoprotein. HeLa cells make alpha subunit in serum-free media with growth factor supplementation, at rates up to 80 percent of those in standard medium containing fetal calf serum.

BIBLIOGRAPHIC REFERENCES: Dean, D. J., Weintraub, B. D., and Rosen, S. W.: De novo synthesis and secretion of heterogeneous forms of human chorionic gonadotropin and its free alpha subunit in the human choriocarcinoma clonal cell line, JEG-3. *Endocrinology* 106: 849-858, 1980. Neuwald, P. D., Anderson, C., Salivar, W. O., Aldenderfer, P. H., Dermody, W. C., Weintraub, B. D., Rosen, S. W., Nelson-Rees, W. A., and Ruddon, R. W.: Expression of oncodevelopmental gene products by human tumor cells in culture. *J. Natl. Cancer Inst.* 64: 447-459, 1980.

72. HUMAN SERUM DNA-BINDING PROTEINS

Pursons, R. G., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Biological Markers Program, P.O. Box B, Frederick, Maryland, 21701, United States of America

OBJECTIVES: The objectives of this project are to examine human serum and plasma DNA-binding proteins to determine whether there are unique malignancy-associated species that may be used as tumor markers. Newly identified species will be isolated, characterized, and evaluated in appropriate clinical studies for their usefulness as tumor markers.

APPROACH: Human serum DNA-binding proteins are obtained by affinity chromatography on DNA-cellulose. Studies are aimed at resolving minor species that are present only in cancer sera from the major DNA-binding proteins that are present in normal sera. This is accomplished through the combination of preparative chromatographic methods (salt and pH elu-

tion steps on DNA-cellulose, immunoadsorption techniques, and the use of high resolution electrophoretic systems (one- and two-dimension). The malignancy-associated serum DNA-binding proteins are then evaluated in clinical studies to determine their efficacy as tumor markers.

PROGRESS: Our laboratory has recently reported the presence of a second human serum DNA-binding protein, which we have termed MAD-2. This malignancy-associated serum DNA-binding protein has been purified and identified as a fragment of fibronectin. A panel of 162 sera, consisting of samples from normal individuals, patients with various nonmalignant diseases, and patients with malignant diseases, has been assayed for both total fibronectin and MAD-2. These studies have established that both MAD-2 and total fibronectin levels are elevated in serum from patients with various malignant diseases, including carcinomas of the breast, lung, testis, and ovary. Elevated MAD-2 and fibronectin levels are found in sera from some patients with nonmalignant inflammatory diseases, although at a lower frequency than those found for patients with malignant diseases.

73. DNA-BINDING PROTEINS IN HUMAN SERUM

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Human sera contains a unique set of proteins with affinity for DNA. Initial characterization of these DNA-binding proteins has indicated their universal occurrence and alterations of these proteins have been found in malignant diseases. The primary foci of this research are the complete biochemical, immunological, and genetic study of the major species of DNA-binding proteins resident in normal human sera and the identification and characterization of the DNA-binding proteins with potential as tumor markers or indicators of other disease states. Current studies will emphasize the nature of the DNA-protein interactions specific to the serum DNA-binding proteins with emphasis on the localization of the actual DNA-binding domains; such studies are ultimately directed toward a correlation of the DNA-binding ability of these proteins with physiological functions. Secondly, there will be a continued evaluation of the diagnostic or prognostic potential of serum nucleic acid-binding proteins. Thirdly, studies will be continued to correlate nucleic acid-binding proteins of nuclear origin with their serum counterparts.

BIBLIOGRAPHIC REFERENCES: Zardi, Luciano, Annalisa Siri, Barbara Carmemolla, Leonardo Santi, William D. Gardner, and Sallie O. Hoch, 1979. Fibronectin: A chromatin associated protein? *Cell*, 18:649. Gardner, William D., Jeanne A. Haselby and Sallie O. Hoch, 1980. Identification of a major serum DNA-binding protein as factor B of the alternative complement pathway. *J. Immunol.*, in press.

74. PROTEIN SAA IN NEOPLASTIC DISEASE

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The objectives of this research proposal are to determine serum levels of protein SAA in neoplastic diseases, relate these data to diagnosis and prognosis, and define the relationship between this protein and the immune dysfunctions noted in cancer. Specifically studies are planned to determine if SAA is synthesized by neoplastic cells or is a factor synthesized as a reaction to a neoplasm. SAA is a serum alpha globulin known to be present in increased amounts in inflammatory diseases as well as in cancer. It has previously been shown to suppress the in vitro formation of antibody by CBA/J murine splenic lymphocytes.

Sera will be collected from patients with cancer of lung and gastrointestinal tract and SAA levels determined by radioimmunoassay. SAA levels will be correlated with histologic type of tumor, presence of metastases, response to therapy and recurrence of tumor. The relationship of SAA levels to abnormalities in lymphocyte function will be determined by measuring the PBL response to mitogens and the in vivo production of antibodies to tetanus toxoid in these patients.

The effect of SAA on the in vitro anti SRBC response of human peripheral blood lymphocytes (PBL) will be characterized using a system currently in use in this laboratory. PBL are cultured with SRBC antigen and the plaque forming cells enumerated by the Jerne assay four days later. Purified human SAA will be added to cultures of PBL in varying concentrations and the effect on response to SRBC measured by hemolytic plaque assay. The objective of these studies are: 1) to further characterize the suppression of immune function by SAA, 2) correlate this with the in vivo SAA level, and 3) determine whether neoplastic cells synthesize SAA.

BIBLIOGRAPHIC REFERENCES: Benson, M.D., and Cohen, A.S.: Serum amyloid A protein (SAA) in amyloidosis, rheumatic and neoplastic diseases. *Arthritis Rheum.* 22:36-42, 1979. Benson, M.D.: Synovial fluid SAA levels in arthritis. *Clin. Res.* 27:141A, 1979 (Abstract).

75. STUDY OF POLAROGRAPHIC CATALYTIC WAVES IN SERUM

Jordan, W. P., Lyons, H., U.S. Veterans Admin., Dept. of Medicine & Surgery, Medical Center, Urological Section, 1030 Jefferson Ave., Memphis, Tennessee, 38104, United States of America

The major objective of this research is the identification and characterization of the serum protein which is associated with the production of a catalytic polarographic wave in the presence of cobalt ions. This protein has been reported in the serum of persons with a diagnosis of various types of cancer.

The first phase of the research plan involves the establishment of a sensitive procedure to measure the catalytic wave height by means of Differential Pulse Polarography. This phase will involve a study of the kinetic factors affecting the wave such as reaction rates, pH, ionic strength, current density, etc. Model proteins of known structure will be used in this phase.

The procedure established above will be used to establish norms for a healthy, volunteer population and also to study the occurrence of the catalytic wave in blood from patients with systemic disorders of a non-cancerous nature. Blood from patients with a diagnosis of cancer will also be examined.

All serum samples which give a statistically significant enhancement of the catalytic wave will be subjected to extensive analysis so as to separate the protein or proteins associated with the enhancement and to characterize these proteins as far as possible.

76. ABNORMAL METABOLISM OF CANCER PATIENTS

Rudman, D., Chawla, R. K., Emory University, School of Medicine, Dept. of Medicine, 1364 Clifton Rd. N.E., Atlanta, Georgia, 30322, United States of America

This project is concerned with the isolation and characterization of cancer-related proteins which are excreted in the urine by patients with advanced cancer. For each purified protein, a specific radioimmunoassay (RIA) is developed. The usefulness of each new RIA in the diagnosis of neoplastic disease is then studied.

BIBLIOGRAPHIC REFERENCES: Rudman, D., Chawla, R.K., Nixon, D.W., Vogler, W.R., Keller, J.W., and MacDonell, R.C. Proteinuria with disseminated neoplastic disease. *Cancer Research* 39: 699-703, 1979. Fine, R.M., Chawla, R.K., Kutner, M., Rudman, D. Accumulation of urinary cancer related glycoprotein, EDCl, in psoriasis. *J. Invest. Derm.* 73: 264-265, 1979.

77. SERUM HIGH DENSITY LIPOPROTEIN-2 IN CANCER PATIENTS

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This is part of a broader project. A summary of this subproject is not available.

78. PURIFICATION OF CANCER-ASSOCIATED PEPTIDES FROM URINE

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OBJECTIVES: To isolate and identify cancer-associated peptides, especially from patients with gynecologic cancer, and set up specific methods for their determination.

APPROACH: Concentration of urine with a new method which allows recovery of peptides with a molecular weight of

1000-10000 (and larger), fractionation of the peptides by gel filtration, and removal of normal peptides and proteins from the fractions by negative affinity chromatography using immunoabsorbents against normal proteins. The fractions thus obtained will be used for immunization. The antisera are tested by crossed immunoelectrophoresis; cancer-associated peptides and proteins are purified and used to set up immunochemical determination methods.

PROGRESS: We have prepared antisera and immunoabsorbents against normal urinary protein fractions. Urine from one cancer patient (ovary) has been partially purified. We have prepared an antiserum against this peptide. By using the antiserum for immunodiffusion we can detect the peptide in 1/4 of all patients with ovarian cancer. We are developing a sensitive radioimmunoassay to be able to study the occurrence of the peptide in different diseases, in pregnancy and in normal subjects.

79. VASOACTIVE INTESTINAL AND LUNG POLYPEPTIDES - TUMOR

Said, S. I., Mutt, V., Bodanszky, M., University of Texas, Health Science Center at Dallas, Southwestern Medical School, Dept. of Internal Medicine, 5323 Harry Hines Blvd., Dallas, Texas, 75230, United States of America

This proposal deals with the relationship of three newly identified polypeptides, vasoactive intestinal peptide (VIP) and two vasoactive lung peptides, to a number of malignant tumors. Using a sensitive and specific radioimmunoassay (RIA), VIP has been shown to occur normally in the gastrointestinal and nervous systems, and in elevated concentrations in these tumors: pancreatic (islet-cell) adenomas, pheochromocytoma, neuroblastoma, medullary thyroid carcinoma, and bronchogenic carcinoma. The clinical syndrome in common among these tumors was that of watery diarrhea (pancreatic cholera), accompanied in some instances by hyperglycemia, hypercalcemia, hypokalemia and episodic flushing. One vasoactive lung peptide, extractable from normal lung, is related to VIP, cross-reacting with it in the RIA. The other vasoactive lung peptide is spasmogenic and does not cross-react with VIP. Determination of the prevalence of secretion of these peptides in the presence of malignant disease may provide useful clues to the early diagnosis, better management and, possibly also, the pathogenesis of these lesions.

BIBLIOGRAPHIC REFERENCES: Frandsen, E.K., Krishna, G.A., and Said, S.I. Vasoactive intestinal polypeptide promotes cyclic adenosine 3',5'-monophosphate accumulation in guinea pig trachea. *Brit. J. Pharmacol.* 62:367-369, 1978. Said, S.I. and Zfass, A.M. Gastrointestinal Hormones. *Disease-A-Month*, 24:1-40, 1978.

80. PEPTIDE MARKERS OF CANCER DERIVED FROM TUMOR-ASSOCIATED PROTEASES

Putterman, G. J., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Biological Markers Program, P.O. Box B, Frederick, Maryland, 21701, United States of America

Malignant tumors have been shown to contain elevated levels of proteases. The objective of this study is to develop markers of cancer based on protease degradation products that appear in human physiological fluids. Two approaches are being used. One approach assumes that a tumor-associated plasminogen activator would generate plasmin and that products of plasmin(ogen) autolysis would appear in physiological fluids of cancer patients. These peptides have either been chemically synthesized or isolated from purified plasminogen in our laboratory and radioimmunoassays (RIAs) are being established for them. Cancer patients' fluids will be assayed using these RIAs. A second approach consists of looking for low molecular weight peptides (less than or equivalent to 30,000 Mr) in cancer patients' fluids using the highly sensitive and selective techniques of high pressure liquid chromatography and counter-current chromatography. The uniqueness of isolated peptide fragments to the cancerous state will be ascertained by examining fluids of normal and non-malignantly diseased subjects.

81. PATIENT STUDIES USING MSA (MULTIPLICATION STIMULATING ACTIVITY) RADIORECEPTOR ASSAY

Hendricks, C. M., Gorden, P., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, Na-

tional Inst. of Arthritis Metabolism & Digestive Diseases, Clinical & Cellular Biology Section, 9000 Rockville Pike, Bethesda, Maryland, 20205, United States of America

MSA (Multiplication stimulating activity) is an insulin-like peptide which reacts with its own receptor and the insulin receptor. MSA is elevated in about 40 percent of patients with hypoglycemia and non-islet cell tumors. Patients with hemangiopericytoma have elevated circulating MSA levels.

82. C-REACTIVE PEPTIDE IN DIAGNOSIS OF INSULINOMA OF PANCREAS (HUMAN)

Service, F. J., Mayo Foundation, 200 1st St. S.W., Rochester, Minnesota, 55901, United States of America

This is part of a broader project. A summary of this subproject is not available.

83. BIOCHEMICAL MARKERS IN CANCER

Malkin, A., Kellen, J. A., Sunnybrook Hospital Medical Centre, Dept. of Clinical Biochemistry, 2075 Bayview Ave., Toronto, Ontario, M4N 3M5, Canada

OBJECTIVE: The objective of this study is to identify optimal biochemical marker combinations in various solid tumors, which would enable us to stratify cancer patients into more homogenous prognostic groups for clinical trials, facilitate the monitoring of treatment and lead to more individualized therapy. The following markers are currently under investigation: Carcinoembryonic antigen, alpha-fetoprotein, placenta-like alkaline phosphatase, chorionic gonadotropin and the acute phase proteins, C-reactive proteins, acid glycoprotein and alpha-1 anti-trypsin. Furthermore, the prognostic value of the trend as expressed in at least four consecutive CEA determinations during a period of at least 6 months is being analyzed in patients with cancer of the breast.

PROGRESS: Our results so far indicate that single markers lack both specificity and sensitivity; different marker combinations are required for different tumors. In primary breast cancer, post-operative patients with elevated CEA levels are more prone to develop recurrent disease; this appears to be independent of nodal status and stage.

84. EVALUATION OF THE CLINICAL SIGNIFICANCE OF TUMOR MARKERS IN THE SERUM OF PATIENTS WITH TUMOR OF THE TESTIS

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OBJECTIVE: To test the usefulness of a panel of tumor markers in order to diagnose as early as possible a developing carcinoma of the testis, preferably before it becomes clinically manifest, and to evaluate the markers' value in monitoring patients who are undergoing chemotherapy. Step 1: Evaluation of the clinical significance of markers known for other carcinomas. Step 2: Search for new tumor (testis)-specific antigens.

APPROACH: The following potential markers are included in the panel: AFP, CEA, LDH, HCG, SPI, alpha-1-antitrypsin and alpha-2-macroglobulin. Their serum values will be established longitudinally before and after operating on patients who are under control with or without chemotherapy. The other part of this phase of the study will be devoted to extraction of tumor (testis) tissue in order to raise antisera towards specific oncofetal antigens, as has been described for liver (AFP), colon (CEA), lung tumors and malignant melanoma.

PROGRESS: On the basis of our study of 80 patients with non-seminoma testicular tumors, the following can be concluded: 1. The complete disappearance of markers (AFP and HCG) after chemotherapy indicated histological absence of tumor in only 30%. 2. The pattern of AFP and/or HCG half-life during chemotherapy correlated with histological evidence of the presence or absence of residual tumor in only 60%. 3. LDH can be used as a reliable marker. 4. Antitrypsin and alpha-2-macroglobulin do not give any additional information. 5. CEA levels in this group of patients always remained below 5 ng/ml, with the exception of one in whom the level increased parallel with a bleomycin pneumonitis; CEA could be measured in the cystic fluid of metastases in 10 patients. 6. SPI was found elevated in 14 patients; the SPI and HCG profiles were parallel.

85. BIOLOGIC TUMOR MARKERS IN THE CELLS AND SERA OF PATIENTS WITH UROLOGIC CANCERS

Javadpour, N., Lippert, M., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Surgery Branch, 9000 Rockville Pike, Bethesda, Maryland, 20205, United States of America

Techniques have been developed to localize and quantitate biologic tumor markers in the cells and sera of patients with urologic cancers. These markers include human chorionic gonadotropin, alpha-fetoprotein, pregnancy specific beta 1 glycoprotein (SP1), different isoenzymes of lactic dehydrogenase (LDH), ABO(H) antigens, and steroid receptors. These markers have been correlated with the histology, grade, stage, and management of patients with urologic cancers. Experimental animal models of cancer induction for the kidney, bladder, prostate, and testis have also been developed. These animal models are being utilized to study the role of cytoreductive surgery, chemotherapy, and immunotherapy of these urologic cancers. Athymic mice have been utilized for xenograft of testicular tumor that produces markers. By utilizing specific antibody labeled with a gamma-emitting radioisotope such as ¹³¹I, the role of radioimmunodetection and radioimmunotherapy is being investigated in testicular and prostatic cancer.

BIBLIOGRAPHIC REFERENCES: Javadpour, N., Woltering, E.A., and Soares, T.: Simultaneous measurement of tumor cytosol and peripheral serum levels of HCG and AFP. *Invest. Urol.*, in press (June 1980). Javadpour, N.: Radioimmunoassay and immunoperoxidase of pregnancy specific beta 1 glycoprotein (SP1) in certain testicular cancer. *J. Urol.*, in press (April 1980).

86. SERUM AND LIVER ALPHA-1-ANTITRYPSIN (ALAT)

Clerc, M. J., Houvet, D., Université d'Abidjan, Faculté de Médecine, Laboratoire de Biochimie Médicale, B.P.V. 166, Abidjan, Ivory Coast

The presence of liver PAS-positive amyloid digestible globules in primary liver cancer cases on the Ivory Coast is being investigated. A minimum of 30 new cancer cases per annum is expected. Serum ALAT will be determined by radial immunodiffusion in cases and in an equal number of controls. ALAT phenotypes will be determined by electrofocusing on starch gel and on polyacrylamide gel amphotolins. PAS-positive globules will be extracted and purified. It is also intended to start an investigation of amylases (beginning 1980-1981) and lipoproteins (HDL ADL) in 1980 (preparation, ultracentrifugation, electrophoresis), definition (study of lipidic constituents), apoproteins (PAGE typing)).

C. HORMONE MARKERS AND RECEPTORS IN CANCER DIAGNOSIS AND PROGNOSIS

1. ESTROGEN

87. CYTOCHEMICAL STUDIES ON HORMONAL RECEPTORS IN BREAST CANCER

Tremblay, G., Shyamala, G., Buell, R. H., McGill University, Inst. of Pathology, Dept. of Pathology, 3775 University St., Montreal, Quebec, H3A 2B4, Canada

OBJECTIVE: Biochemical assays of mammary carcinoma tissue for estrogen receptor (ER) provide quantitative data allowing for the prediction of patient response to hormonal therapy but provide little information on the distribution of ER and fail to predict the observed clinical response in about one-third of patients. Recently, cytochemical techniques were developed demonstrating a heterogeneous population of ER positive and negative tumor cells within many mammary carcinomas. This cellular diversity, not evident in biochemical assays, suggests a partial explanation for unexpected therapeutic failures. The object of this project is to develop techniques which will permit the differentiation on tissue sections of ER positive and negative cells in human mammary carcinomas. Special emphasis will be placed on the distribution of ER in atypical (pre-cancerous) lesions and the various histologic patterns of primary breast carcinoma. The long range plan of the project is a correlation of the cytochemical studies, biochemical assays and the biologic evolution of the carcinoma.

APPROACH: To identify ER positive and negative cells in tissue sections, dry-mount or thaw-mount autoradiography

and a peroxidase/anti-peroxidase immunocytochemical technique will be used. The accuracy of the methods will first be verified by studying the distribution of ER in the uterus of BALB/c mice after in vitro incubation with or without specific inhibitors and comparing this with known results obtained from in vivo injection of 3H-estradiol. Once characterized, the methods will be applied to a study of human mammary carcinoma.

PROGRESS: The techniques of dry-mount and thaw-mount autoradiography have been successfully used to demonstrate ER positive cells in the uteri of BALB/c mice after in vivo injection of 3H-estradiol. In vitro incubation schemes are being tested which yield results comparing favorably with those observed after in vivo injections. Preliminary results from the incubation of several human breast cancers have also been obtained. For the immunocytochemical technique, the antigen 17-beta-estradiol-6-(O-carboxymethyl) oxime-bovine serum albumin has been synthesized and used for immunization of rabbits. The antisera obtained are being tested for titre and cross-reactivities. In addition, experiments are being done to localize ER in tissue previously studied by autoradiography.

88. HORMONE RECEPTORS AND ACTION IN HUMAN BREAST CANCER

Fazekas, A. G., MacFarlane, J. K., Montreal General Hospital, Surgical Laboratories, 1650 Cedar Ave., Montreal, Quebec, H3G 1A4, Canada

OBJECTIVE: To predict the response to endocrine ablation or additive hormone therapy with greater accuracy in advanced breast cancer.

APPROACH: The hormone receptor profile for a number of steroid hormones is determined in primary and metastatic breast cancer. Methods for the characterization and quantitative measurement of estradiol, cortisol, dexamethasone and progesterone receptors are routinely used. In addition, the nuclear binding of estradiol is studied. The presence or absence of these receptors is correlated to responses to endocrine treatments.

PROGRESS: Estradiol receptors were detected in 50% of 1,300 cases in significant amounts, in the cytosol of the tumors. Nuclear binding was found in 80% of these cases, the rest was without detectable translocation of estradiol. Glucocorticoid receptors were found in 23% of all cases, while a transcortin-like cortisol binding protein was detected in over 95% of all cases studied. Approximately one-third of cases show progesterone binding activity. Studies indicate that some tumors have receptors for a number of steroid hormones indicative of multiple hormone dependency. A significant proportion of ER positive tumors show no nuclear binding.

89. HORMONAL STUDIES IN CARCINOMA OF THE BREAST

Ospina, J. E., de Baez, N. E., de Pardo, C., de Murcia, T., Instituto Nacional de Cancerología, Sección de Biología Experimental, Apartado Aéreo #17.158, Bogotá, DE, Colombia

OBJECTIVES: a) To establish the hormonal sensitivity or dependence of malignant neoplasms of the mammary gland through the determination of specific estrogen and progesterone receptors; b) to establish the correlation, if any, between the histopathology, ultrastructural characteristics, and estrogen and progesterone receptor levels in the different types of mammary neoplasms; c) to investigate the correlation that might exist between estradiol levels in urine, tumoral histopathology, degree of hormone dependence and tumor aggressivity.

MATERIAL: Tumor biopsies and urine from patients with confirmed diagnosis of breast cancer.

METHODS: a) For determination of hormonal receptors, Liskowski-Rose method and Horwitz-McGuire method; b) for determination of estradiol in urine, clinical assay kits designed by Travenol Laboratories specifically for radioimmunoanalysis; c) electron microscopy - inclusion in epoxy resins, gradual dehydration with alcohol, section with Porter-Blum MT2 ultramicrotome, staining with lead citrate and uranyl acetate; 'JEOL' electron microscope, type JEM 7A; d) cell cultures, started by enzymatic digestion of tumor or sloughing off of cells. (Translated)

90. HISTOCHEMICAL METHODS FOR RECEPTORS IN BREAST CANCER

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Estrogen and progesterone receptors are known to be valuable in predicting endocrine responsiveness and aggressiveness of breast cancers. Histochemical detection of these receptors in the heterogeneous cell populations of most tumors would offer new information on heterogeneity as well as making the receptor determination much faster and cheaper and more sensitive and also opening new possibilities for basic receptor research. We have already shown that existing histochemical receptor methods probably do not actually detect receptors. Here we propose to develop valid cytochemical receptor methods by synthesizing new more stable steroid-fluorescein conjugates and subjecting them to rigorous testing. We also propose to develop new immunocytochemical receptor methods based on specific monoclonal antibodies against the receptors themselves rather than against their ligands. Partial receptor purification by new affinity chromatography reagents will be applied to both estrogen and progesterone receptors from MCF-7 human breast cancer cells, followed by preparation of antibodies by the hybridoma technique and the use of these antibodies for receptor detection in frozen sections from breast cancer specimens.

91. COMPARATIVE STUDY OF ESTROGEN RECEPTOR ACTIVITY IN MAMMARY CANCER BY RADIORECEPTOR ASSAY AND FLUORESCENCE MICROSCOPY

Remmele, W., Frank, K., Heine, M., Heicke, B., Municipal Hospital, Dept. of Pathology, Schwalbacher Str. 62, D6200 Wiesbaden, Federal Republic of Germany

OBJECTIVE AND APPROACH: The purpose of the project is to compare the results of estimation of steroid receptor activity by radioreceptor-assay (Prof. Heicke, Univ. of Mainz-Bioscientia Ingelheim) and by the fluorescence microscopy method of Lee (Amer. J. Clin. Path. 70: 197-203, 1978). The method of Lee is used as originally described as well as in a modified way developed in our laboratory.

PROGRESS: Series of experiments has just started; no data are yet available.

92. ESTROGEN-RECEPTOR ASSAY IN THE MANAGEMENT OF BREAST CANCER

Ross, D. P., University of Wisconsin, Madison Campus, Medical School, Dept. of Human Oncology, 430 N. Randall Ave., Madison, Wisconsin, 53706, United States of America

This is part of a broader project. A summary of this subproject is not available.

93. ESTROGEN RECEPTOR (ER) IN NORMAL AND TUMOR TISSUE OF THE BREAST - CORRELATION WITH CLINICAL AND PARA-CLINICAL PARAMETERS

Rivadeneira, J. H., Gomez, C. P., Guzman-Patrucco, C., Rabago, M., Instituto Mexicano del Seguro Social, Dept. of Pathology, Av. Cuauhtemoc No. 330, c/o Hsp. Oncol.-Ctr. Med. Nac., Mexico City 7, Distrito Fed., Mexico

The results are presented in the following order: type of tumor, number of tumors of a given type, percentage of ER positive tumors, mean value expressed as fm/mg/cp: Malignant tumors: not otherwise specified - 65, 72, 93.19; medullary - 11, 45, 52.80; tubular - 2, 100, 52.00; lobular - 5, 40, 58.00; and c. phyllodes - 3, 100, 22.67. Benign tumors and diseases: fibroadenoma - 16, 38, 43.80; cystic mastopathy - 5, 60, 51.70; c. phyllodes - 3, 67, 110.50.

94. ETIOLOGIC AND PROGNOSTIC FACTORS IN BREAST CANCER

Wotiz, H. H., Boston University, School of Medicine, Dept. of Biochemistry, 80 E. Concord St., Boston, Massachusetts, 02215, United States of America

The objectives of this program-project are two-fold, both aspects being relevant to breast cancer. The first of these is an intensive study of biochemical factors underlying certain possible etiologic factors of this disease. Particularly, we will investigate cellular and systemic factors relevant to the 'Bulbrook theory' and the 'estriol theory' and compare these with a new concept intent on reconciling these apparently divergent theories. At the same time, aided by the availability of the appropriate patient material, prognostic aspects relative to proposed

treatment modalities will be examined. Thus we plan to compare therapeutic predictions obtained with the 'discriminant factor' and with the 'estrogen receptor assay,' as a means of differentiating hormone dependent from independent tumors. In addition, we will proceed with the development of the immunofluorescent technique to make this assessment procedure more readily available.

95. STEROID BINDING IN BREAST CANCER BY IMMUNOFLUORESCENCE

Gaetjens, E., Pertschuk, L. P., Kim, D. S., Brigati, D. J., Carter, A. C., State University of New York, Downstate Medical Center, School of Medicine, Dept. of Pathology, 450 Clarkson Ave., Brooklyn, New York, 11203, United States of America

A successful immunofluorescence technique for detection of estrogen receptor in human breast cancer has been developed. The method is based on the use of an estradiol polymer bound to the specific receptor on frozen sections of tumor. The polymer acts as a large antigen target for subsequent binding of antibody and labeled conjugate. We propose to synthesize water soluble polymers of progesterone, testosterone and glucocorticoid hormones suitable for use in immunofluorescence studies designed to detect specific receptor for these steroids in human mammary carcinoma. Results obtained by immunofluorescence will be compared with those of conventional biochemical assay and correlated with clinical response to therapeutic hormonal manipulations.

BIBLIOGRAPHIC REFERENCES: Pertschuk, L.P., Zava, D.T., Gaetjens, E., Macchia, R.J., Brigati, D.J. and Kim, D.S.: Detection of androgen and estrogen receptors in human prostatic carcinoma and hyperplasia by fluorescence microscopy. Res. Comm. Chem. Pathology and Pharmacology, 22, 427, 1978.

96. BIOLOGIC MARKERS IN BREAST CANCER PATIENTS

Schwartz, M. K., Memorial Hospital for Cancer & Allied Diseases, 1275 York Ave., New York, New York, 10021, United States of America

OBJECTIVES: Determine the concentrations of a selected group of constituent in breast tissue obtained at mastectomy and assay for these same substances in the serum of the patients obtained before their surgery and then sequentially on a scheduled basis over a period of time. Our aim is to find a constituent in cancerous breast tissue that is present in a concentration different from that in normal breast tissue and then to try to establish if changes in these concentrations in the breast tissue are reflected in the serum and whether the course of the disease is reflected in the serum pattern. We are now routinely obtaining in our laboratory breast tissue for analysis of estrogen receptor protein. It is our aim to determine in the cytosol and pellet of these tissues a variety of substances whose assays are in use in our laboratory. In addition, we can make available specimens for analysis in other laboratories.

97. STUDY OF SOME RELATIONSHIPS BETWEEN ESTROGEN METABOLITES AND BREAST CANCER

Cohen, S. L., University of Toronto, Dept. of Obstetrics & Gynecology, Medical Sciences Bldg., Toronto, Ontario, M5S 1A8, Canada

OBJECTIVES: a) To determine a possible relationship between breast cancer and the occurrence of 2-hydroxy (catechol)-estradiol in the urine. Preliminary observations tend to indicate such a relationship. b) To determine the nature of the estrogen bound to the nuclear receptors of breast cancer tissue. Preliminary observations indicate that it is either estriol or 2-hydroxy-estradiol (our conditions of assay did not exclude either of these).

APPROACH: a) To carry out our 'profile' assay technique (Cohen et al., Steroids 32, 279, 1978) on urines from patients with breast cancer and a wide variety of controls: normal cycling women; post-menopausal, less than 5 years, 5-10 years, and more than 10 years; women of all ages being subjected to other surgeries. b) To have the catechol estrogens assayed by the specific mass-spectrometric technique of Rosenberg (Hamilton). c) To assay the catechol estrogens by radioimmunoassay techniques (3 different groups of workers have described such a technique).

PROGRESS: April 1-August 31, 1979: a) We have assayed 15 urines (every other day) from the complete 28-30 day cycles of 3 young women. The assays are presently being checked for me by Dr. Diemann of the Mass. Inst. of Tech. in Boston, by mass-spectrometry. b) An organic chemist (Pauline Zvirsky) is well into the preparation of large quantities of the 3 catechol estrogens for use in the preparation of antibodies. c) I am about to hire someone for the tissue work. Since tissue estrogen assays involve assays which are in the 10 to the minus 6th to the minus 9th power of those in urine, we do not like to do both assays in the same room. So we have arranged to have the tissue assay work conducted in Dr. Mobb's laboratory. The NCI Grant does not permit payment of a technician for more than 1/2 time. So I am hiring someone for 1/2 year on the current grant and 1/2 year on next year's grant. She (he) will thus be able to work full time on the project.

98. ESTRADIOL SENSITIVE MAMMARY CANCER AND LOCALIZATION WITH 197 HG-LABELLED 4-MERCURY-ESTRADIOL

Doorenbos, H., Oldhof, J., Woldring, M. G., Janssens, J., Pratt, J. J., Feenstra, A., University Hospital of Groningen, Dept. of Nuclear Medicine, Oostersingel 59, 9713EZ Groningen, Netherlands

OBJECTIVE: The in vivo localization of breast cancer containing estrogen receptors with 197 Hg-labelled mercury-17-beta-estradiol.

APPROACH: The synthesis of 197 Hg-labelled mercury-17-beta-estradiol with a high specific activity on a very small scale, and the characterization of the interaction of the product with the cytoplasmic and nuclear estrogen receptor.

99. STUDIES ON ESTRADIOL AND PROGESTERONE RECEPTORS IN MALIGNANT TUMORS WITH SPECIAL REFERENCE TO BREAST CANCER (ABBREVIATED)

Norgren, A., University Hospital of Lund, Dept. of Lung Medicine/Research, EB-blocket, S22185 Lund, Sweden

OBJECTIVES: 1) Thorough testing of currently used methods in receptor research with the aim to improve, for instance, yield of receptor, and to make possible the estimation of total concentration of cytosol receptor protein. 2) Elaboration of experimental biological test systems for the study of control mechanisms for receptor synthesis. 3) Study of importance of the different receptor profiles in prospective clinical breast cancer trials with regard to prognosis of the disease and the effect of adjuvant therapy. 4) Estimation of hormone receptor levels in hormone dependent malignant tumors other than breast cancer.

100. STEROID RECEPTORS AND INDUCIBLE PROTEINS IN BREAST TUMORS

Duffy, M. J., O'Connell, M., St. Vincents Hospital, Dept. of Nuclear Medicine, Elm Park, Dublin, Ireland

OBJECTIVE: The aim of this project is to determine if estradiol inducible proteins are better markers for hormone dependent breast cancer than the estradiol receptor alone.

APPROACH: Estradiol receptors and the estradiol inducible protein (peroxidase and progesterone) receptors are being measured in both normal animal tissues and breast tumors. The correlation between the presence of these parameters in human breast cancers and response to endocrine therapy is being investigated. It is hoped to determine if the presence of the inducible proteins adds to the predictive ability of the estradiol receptor.

101. STEROID HORMONES IN BREAST CANCER

Hochberg, R. B., St. Lukes - Roosevelt Hospital Center, Amsterdam Ave. & 114th St., New York, New York, 10025, United States of America

Several aspects of steroid biochemistry as they are related to hormonally dependent tumors, especially breast cancer, are to be studied. Our recent identification of a new class of steroids, non-polar lipoidal derivatives in steroidogenic tissue, has been extended and we have isolated a lipoidal derivative of estradiol that has the chemical structure of the non-polar group covalently linked to estradiol. These studies will be performed by radiochemical techniques in conjunction with purification by high pressure liquid chromatography and identification by gas chromatography/mass spectroscopy, as well as other classical

methods. Further experiments will also be carried out to determine the biochemical events involved in the synthesis of this substance as well as its physiological role. The relationship of the lipoidal estrogen to estrogen responsive tissues and the estrogen receptor as well as the endogenous concentration of this substance will be explored. Our success in synthesizing 1 alpha-125I-estradiol, a gamma-emitting derivative of estradiol, which binds with high affinity and specificity to the estrogen receptor will be fully expanded. While the apparent specific activity, greater than 100 Ci/mM, of the iodinated estrogen is now more than adequate for usual receptor studies, attempts are being made to synthesize carrier free tracer (greater than 200 Ci/mM) for increased sensitivity and for use with small clinical samples. The material now available is being used to develop an assay for the detection of estrogen receptor in breast tumors. 131I labelled estradiol is being prepared for in vivo imaging and as a possible palliative agent. In addition, doubly labelled estradiol (125I and 131I) is to be utilized in vitro for experiments on receptor turnover and subcellular localization. Furthermore, 16-alpha-125I-estradiol has been found to bind to antibodies formed against estradiol haptens conjugated at positions C-3, C-6 and C-17 of the steroid nucleus to bovine serum albumin. Sensitive radioimmunoassays for estradiol are being developed. The exchange technique used for the synthesis of the iodinated estrogen is to be used for the synthesis of other radioiodine labelled steroids that bind to progesterone, androgen, and corticoid receptors.

102. A STUDY OF HORMONE RECEPTORS IN TUMOR TISSUE

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OBJECTIVES: (1) To increase the value of receptor assay in predicting hormone responsiveness of breast tumor by improvement and simplification of our present methods for estimating cytoplasmic oestradiol and progesterone receptors and nuclear oestrogen receptors, and to extend the scope of receptor analysis by inclusion of additional hormone receptors. (2) To continue and extend clinical pilot studies on breast carcinoma to provide information on: (a) receptor status and correlation with hormone therapy, (b) relationship between receptor status and response to cytotoxic chemotherapy, (c) usefulness of receptor profile of primary tumor as a predictor of hormone responsiveness of subsequent metastases, (d) effects of chemotherapy, radiation, hormone and anti-oestrogen treatment on receptor site status, and (e) relationship between receptor status and stage of disease.

APPROACH: Current methods of assaying receptors in general are hampered by inavailability of sufficient tissue on many occasions. An attempt will be made to develop more sensitive methods for the assay of receptor sites based on the availability of specific antibody to the receptor protein: These would include use of fluorescently labelled antibody in tissue slices and radioimmunoassay in cytosol. The first step involves isolation and purification of oestradiol receptor from human tumor tissue, using as starting material RE-c positive primary breast tumor obtained at mastectomy. After characterization, the receptor complex will be used to elicit antibody. Alternative methods which may prove to be more sensitive e.g., isoelectrofocusing in various matrices, are also being examined.

PROGRESS: The addition of nuclear receptor (RE-N) assay appears to reinforce the greater precision of prediction of response to hormone therapy in advanced breast cancer; this method involves measuring progesterone receptor as well as oestradiol receptor in the tumor cytosol.

Patients with RE-c positive RP-c positive primary tumors have been shown to have a longer disease-free interval than patients with RP-c negative tumors, irrespective of whether these were RE-c positive or RE-c negative. Both cytoplasmic assays and more recently the additional RE-N assay have been carried out on the tumors from 400-500 patients at mastectomy who are being followed for recurrence and subsequent response.

103. ESTRADIOL GLUCURONIDATION AND BREAST CANCER

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Studies from this laboratory by Fishman et al (Science 204:1089, 1979) have reported that women at familial high risk for breast cancer have an abnormal pattern of urinary estrogen excretion: whereas low-risk women show a marked rise of the excretion of estradiol glucuronide and estrone glucuronide during the periovulatory and luteal phases, high-risk women show less rise above follicular excretion levels, so that a significant difference in estrogen glucuronide excretion between the two groups appears during the later phases that is not present during the earlier phase. To explain these findings, it was postulated that high-risk women have an innately limited capacity to form glucuronide conjugates of estrogens, and that this capacity is exceeded during the periovulatory and luteal phases when the estradiol secretion rate rises sharply above the baseline follicular level. The objective of the present study is to compare the estradiol-glucuronidating capacity of high-risk and low-risk women directly by measuring the steady-state urinary excretion of estradiol glucuronide that is achieved during a six-hour, constant-load intravenous infusion of a supraphysiological amount of estradiol (500 ng/minute). If deficient glucuronidating capacity is demonstrated in high-risk women as a group, this measure could be applied, in a later study, to the investigation of multiple members of high-risk kindreds, in order to determine the risk status of individuals and to establish the pattern of inheritance of risk.

104. ESTROGENS AND BREAST CANCER RISK, RESERPINE AND BREAST CANCER RISK, ESTROGENS AND ENDOMETRIAL CANCER RISK

Henderson, B. E., Gerkins, V. R., MacMahon, B., Cole, P. C., Mayes, D. S., Brown, J., Phillips, R. L., Williams, P. A., Pike, M. C., University of Southern California, School of Medicine, Dept. of Pathology, 2025 Zonal Ave., Los Angeles, California, 90033, United States of America

OBJECTIVES: (1) To determine the role of estrogens given for menopausal symptoms in the development of breast and uterine cancer. (2) To evaluate female hormone levels in female relatives of bilateral breast cancer patients less than 50 years old. (3) To evaluate female hormone levels and dietary practices in high school students of the Seventh-Day Adventist Academies in Los Angeles.

105. ESTROGEN PROFILES IN RELATION TO OVARIAN TUMORS AND UTERINE CANCER

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OBJECTIVES: (a) To determine etiological factors related to estrogen production and metabolism which may predispose to ovarian and uterine cancer. (b) To formulate methods of reducing the incidence of these cancers. (c) To develop hormone screening tests to identify women at risk.

APPROACH: Measurement of several estrogen metabolites in urine and several androgens and estrogens in serum of postmenopausal women with high risk conditions, e.g., atypical hyperplasia of the endometrium and obesity, and compare the values with those seen in postmenopausal cases and controls.

PROGRESS: Women with atypical hyperplasia of the endometrium requiring hysterectomy have higher estrogen values than women with carcinoma of the endometrium; this estrogen is derived from the ovaries and the structures involved are being investigated. Approximately 40-50% of patients with endometrial cancer have higher estrogen values than the controls; this estrogen is not of ovarian origin. A much larger series of cases and controls is now being studied.

106. CLASSICAL & UNUSUAL ESTROGENS AS CONTROL FACTORS OF HORMONE-RECEPTOR & HORMONE-SENSITIVITY IN HUMAN AND EXPERIMENTAL HORMONE-SENSITIVE TUMORS

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Our research aims to correlate estrogen profiles with the hormone sensitivity of human and experimental tumors, in vivo and in vitro. Estrogen hormone profiles, also related to other steroids, are evaluated as plasma rates, urine excretion levels, and ratios with reference to opportunely selected parameters. Plasma rates are studied with RIA tests, and excretion profiles are evaluated through gas-liquid chromatography and mass-spectrometry.

Various approaches are employed: a) comparison/evaluation of steroid profiles and their receptors, used as more reliable parameters of clinical hormone response. The functioning of the receptor mechanism is studied with assays of both cytoplasmic and nuclear receptors of E₂; b) evaluation of steroid profiles as basic factors in hormone status study, in both neoplastic and dysplastic disease; c) study of the correlations between steroid status modifications and clinical response to various therapies (surgical, hormonal, chemo- and radio-therapy), in both hormone-responsive and non-responsive tumors; d) study of incretion, of steroid circulation and excretion rates regulating factors, and identification of some of their biochemical actions, i.e.: 1) definition of classical and unusual metabolite reciprocal regulation; 2) influence of various steroid metabolites on carrier proteins; 3) possible interferences of the unusual metabolite fraction in the regulation of the steroid receptor content (E₂R, PgR, TR and DHTR).

These researches should be carried out on experimental models in vivo and/or on tissue cultures of hormone-dependent human tumors such as MCF-7. All these studies are carried out on the principal tumor types that may be classified as hormone-sensitive: breast, endometrial and prostate tumors.

107. ESTROGEN METABOLISM IN PATIENTS WITH ENDOMETRIAL CARCINOMA - EFFECT OF MEDROXYPROGESTERONE ON THE PROGNOSIS OF ENDOMETRIAL CARCINOMA

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We are investigating the secretory capacity, with regard to various steroids, of the ovaries of postmenopausal women with or without endometrial carcinoma using chemical, enzyme histochemical and ultrastructural methods. The plasma levels are measured in ovarian and cubital veins. The studies are in progress.

This is an old project and is organized together with the Departments of Obstet. and Gynec. of Helsinki and Oulu. The results are now under analysis.

108. STEROID HORMONE RECEPTORS AND CIRCULATING HORMONE LEVELS IN GYNECOLOGICAL MALIGNANCIES

Kaupilla, A., Vihko, R., Janne, O., Syrjala, P., Oulu University Central Hospital, Dept. of Obstetrics & Gynecology, Kajaanintie 51, 90220 Oulu 22, Finland

OBJECTIVE: To determine the cytoplasmic and nuclear receptor binding sites for estradiol and progesterone in endometrial and ovarian malignancies. The clinical value of these results in the treatment of endometrial carcinomas with high-dose progestogens will be evaluated. Also, the possible importance of receptor induction will be evaluated.

APPROACH: The estrogen and progesterone receptor measurements were performed with a dextran-coated charcoal technique and sucrose density gradient centrifugation. Serum estradiol, progesterone, LH, FSH and prolactin levels were determined by radioimmunoassay.

PROGRESS: Until now about 150 gynecological malignancies have been studied. The results have been presented in the 2nd International Meeting on Endometrial Cancer and Related Subjects, London, 30-31st March, 1977 (Abstracts) and in XVI Nordiska Kongressen for Klinisk Kemi och Klinisk Fysiologi, Oulu 28-30.6. 1977 (Abstracts, p. 81). Syrjala, P., Kontula, K., Janne, O., Kaupilla, A., and Vihko, R.: Steroid receptors in normal and neoplastic human uterine tissue. In Gynecological Malignancy. Eds. Brusch, M.G., Taylor, R.W. and King, R.J.B. Bailliere Tindall, London, 1978, in press. R. Vihko was a joint author of chapter Receptors for steroids in human endometrium. In Hormonal biology of endometrial cancer. A series of work-

shops on the biology of human cancer. Report No 8. Eds. Richardson, G.S. and MacLaughlin, D.T., International union against cancer. Technical Report Series, Vol. 42, 63-112, 1978.

This project will continue for 3-5 years.

109. GYNECOLOGICAL CANCER CLINICAL LABORATORY

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Steroid receptor and peroxidase assays will be continued to confirm their hormone regulation in normal endometrium and to evaluate their possible use in prediction of hormone responsiveness of endometrial carcinoma. Antibodies to estrogen receptor will be studied to establish an immunochemical assay for the endometrial estrogen receptor which will be related to binding assays with labeled steroid and also hormone responses.

Additional data are being collected and evaluated to study correlations between: 1) biochemical parameters related to sex steroid hormone receptors; and 2) histopathologic and clinical parameters. A high priority will be to establish ovarian adenocarcinoma culture systems in vitro and in nude mice. The rationale for this latter objective is to learn the growth patterns of the ovarian cancer cells under different experimental conditions, e.g., presence and absence of sex steroid hormones, antiestrogens and chemotherapeutic agents. We also plan to perform uptake studies with 125I-labeled estradiol which has become available in the last year.

We will continue clinical studies of the effects of lithium on blood counts, circulating stem cells, and CSA production in patients receiving chemotherapy. A larger patient population and longer follow-up will be needed to determine the effects of lithium in this setting. We also plan to develop both a long term hematopoietic stem cell assay (Dexter-type) and reliable tumor cell culture capabilities in order to eventually expand our studies of lithium into these areas.

110. THE ROLE OF STEROID RECEPTOR PROTEINS IN DIAGNOSIS AND MANAGEMENT OF BENIGN AND MALIGNANT PROSTATIC DISEASES

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OBJECTIVE: To measure androgen, estrogen and progesterone receptor protein concentrations in the cytosol and nuclear fraction of prostatic tissue in benign prostatic hypertrophy and carcinoma of the prostate.

These data are required in order to characterize malignant tissue and then to identify sub-groups or populations which might be correlated with prognosis and treatment.

The concentration of receptor proteins in benign prostatic tissue not only provides a basis for comparison but may also indicate parts of benign hypertrophy where hormonal methods for growth control could be considered.

111. PROSTATE STEROID RECEPTORS BY FLUORESCENCE MICROSCOPY

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The primary objective of this project is to develop histochemical assays for the detection of estrogen and androgen receptors in human prostate hyperplasia and carcinoma. Results of these assays are to be correlated with independently performed conventional biochemical receptor assays in a double-blind study. Ligand-conjugates composed of 17-beta-estradiol and 17-beta-testosterone bound to bovine serum albumin via a hemisuccinate bridge and labeled with fluorescein are employed. Processed tissues are examined by fluorescence microscopy in conjunction with suitable competitive binding assays.

2. OTHER SEX HORMONES

112. HORMONES AND BREAST CANCER PROSTAGLANDIN BIOSYNTHESIS

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The hypothesis is proposed that prostaglandin biosynthesis by breast cancers is stimulated by prolactin in tumors which are hormonally sensitive. This is based on evidence that prolactin stimulates prostaglandin production in normal murine mammary tissue. This hypothesis predicts the following experimental observations: 1) Prostaglandin concentrations should be higher in hormonally sensitive tumors; 2) prolactin should stimulate prostaglandin production in hormonally sensitive tumors; 3) estrogens should induce prostaglandin synthetase in hormonally sensitive breast tumors; or 4) estrogens should increase the sensitivity of the prostaglandin synthetase system to prolactin in hormonally sensitive tumors. We plan to test these predictions using radioimmunoassay measurements of tissue prostaglandin E2, prostaglandin synthetase and phospholipase A2 activity in human breast tumors which are classified as hormonally sensitive by the presence of estrogen receptors and in DMBA induced rat mammary tumors which have been exposed to altered (elevated and/or lowered) levels of estrogen and/or prolactin. These latter manipulations will be accomplished by injection of estrogen, ovariectomy and injection of prolactin or administration of prolactin elevating or prolactin release inhibiting drugs.

113. GLUCOCORTICOID BINDING PROTEINS IN MAMMARY CARCINOMA - HORMONAL REGULATION AND RELATIONSHIPS WITH SPECIFIC PROTEIN SYNTHESIS

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OBJECTIVE: The aim of this project is to investigate glucocorticoid-protein interactions in mammary carcinoma and in diseases admittedly associated with high risk of breast cancer (gross cystic disease). Evidence for and definition of specific glucocorticoid-recognizing proteins, hormonal regulation of binding, and relationships to synthetic activities of the mammary cell are selected topics to be evaluated.

APPROACH: Measurement (Fazekas and McFarlane, Cancer Res. 37:640-645, 1977, modified) of glucocorticoid binding activities both in experimental (R3230AC, Fischer rat) and in human breast specimens (normal, gross cystic and cancer). Characterization of the binding parameters with respect to different glucocorticoid molecules; comparison to serum and milk binding (corticosteroid-binding globulin, transcortin), and to activities in other tissues (e.g., liver); studies on modulation by different hormonal and non-hormonal treatments; correlation with specific (casein) and/or non-specific (enzymes, Beta2-microglobulin) protein contents in the tumor or in the cyst fluid.

PROGRESS: 1) Experimental carcinoma (R3230AC). Significant amounts of a transcortin-like protein which binds both corticosterone and cortisol have been found in almost all cytosol samples examined (n equals 55). This protein is apparently secreted in the fluid of the tumor cysts, as extremely elevated values were found in this medium. Specific binding for dexamethasone was present in the tumor cytosol in about 30% of specimens. Dexamethasone binding sites were not present in the tumoral fluid. In hypophysectomized animals the pattern of glucocorticoid binding was not different. Pharmacological manipulation of prolactin secretion did not interfere with the binding activities; administration of estrogens did significantly reduce the transcortin-like binding without affecting the dexamethasone binding. 2) Human mammary carcinoma. A remarkable binding activity for cortisol and corticosterone was apparent in about 50% of cytosol samples examined (n equals 24). Dexamethasone binding was detectable in only 20% of specimens and was quantitatively lower with respect to both other molecules. 3) Human gross cystic disease. Relevant quantities of a specific cortisol plus corticosterone binder have been found in about 40% of the cyst fluids examined. (Text Abridged.)

114. HORMONAL FACTORS IN THE ETIOLOGY OF BREAST CANCER

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Results of a prospective study of the island of Guernsey, obtained in this laboratory, have shown that women who subsequently develop breast cancer have, on the average, a subnormal excretion of urinary androgen metabolites. The investigation now in progress is to examine (1) whether in a prospective study this urinary abnormality is reflected in the general circulating levels of plasma androgens, (2) plasma androgen levels in women with increased risk of breast cancer (e.g., race, familial history, age at first birth, etc.) and (3) the general androgen status in women with established breast cancer.

115. ADRENAL C19DELTA5-STERIODS AND HUMAN BREAST CANCER

Adams, J. B., Seymour Munn, K., University of New South Wales, School of Biochemistry, P.O. Box 1, Kensington, N.S.W. 2033, Australia

OBJECTIVES: To test the hypothesis that adrenal C19delta5-steroids may be involved in the etiology of human breast cancer. 5-Androstene-3beta,17beta-diol, which has a high affinity for the estrogen receptor, may be the key compound whereby elevated C19delta5-steroids produced by the adrenal could provoke a continuing estrogenic stimulus. The diol is formed peripherally from dehydroepiandrosterone and the latter is in equilibrium with its 3beta-sulfate. Thus a 'buffer' system exists to maintain blood levels of the diol. Current work is therefore concentrating on 5-androstene-3beta,17beta-diol in experiments involving (1) assessment of concentrations in human mammary tumors along with E1 and E2 relating these to levels of estrogen and progesterone receptors, (2) determination of blood levels in rats bearing pellets of the compound and relating these levels to estrogen responses such as uterine weight gain, and (3) study of its metabolism in MCF-7 cells.

BIBLIOGRAPHIC REFERENCES: Adams, J.B. Archibald, L. and Seymour-Munn, K. Dehydroepiandrosterone and 5-androstene-3beta,17beta-diol in human mammary cytosolic and nuclear compartments and their relationship to estrogen receptor. *Cancer Res.* 40: 3815-3820, 1980.

116. NATURAL HISTORY OF POSTMENOPAUSAL BREAST CANCER

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Population screening for breast cancer (palpation, xeroradiography) of 20,000 women 50-64 years of age, at regular intervals (12-18 months) will enable the investigators to study factors which determine incidence and growth rates of preclinical breast cancer occurring after menopause. Particular attention will be paid to endocrine and nutritional factors. Urine is being collected for determination of 11-deoxy-17-ketosteroids and frozen for future research (prospective study). Body weight and height and skinfolds are being measured. Blood pressure is taken and data on antihypertensive drug treatment and other possible risk factors are being collected. Blood samples are being taken at the second examination. Interim publications appeared in *Screening in Cancer, UICC Techn. Rep. Ser.*, 40, 183-200, 1978, *Brit. J. Cancer*, 39, 688-695, 1979 and *Oncology* 37, 33-36, 1980. Others are to appear in Proceedings of Conferences held at Monaco, Talloires, London (Depca), Cambridge (Int. Un. Nutr. Sc.), Oslo (UICC). A paper on estrogen receptors has been submitted to *Cancer Research* and a doctor's thesis published by Rombach. The screening part of the study will be completed at the end of 1980 but the population will be followed for several years by means of a cancer registry.

117. HORMONAL LEVELS AND HORMONAL RECEPTOR CONCENTRATION IN THE BREAST CANCER - CORRELATION WITH PROGNOSIS AND THERAPY

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OBJECTIVE: The following criteria must be fulfilled for breast cancer patients to be enrolled in our project: age under 70 years; performance status 60; operable tumor; no distant metastases; no previous hormone, chemo- or radiation therapy; at least 6 nodes dissected at surgery.

APPROACH: Seventy-nine patients have been enrolled as of mid-December 1979. They have been randomized into the following groups: GUN 1 - 32 postmenopausal patients with

positive nodes; 18 received Tamoxifen, 14 no additional treatment after surgery. GUN 2 - 15 postmenopausal patients with negative nodes; 5 received Tmx, 10 no additional treatment after surgery. GUN 3 - 24 premenopausal patients with positive nodes; 12 received CMG plus Tmx, 12 only CMF after surgery. GUN 4 - 6 premenopausal patients with negative nodes; 2 received Tmx, 4 no additional treatment after surgery. Five patients had recurrence of the disease (1 skin, 2 skin and lungs, 1 nodes, ICNS). In 4 of the above cases, no receptors for E2 were found in the original tumor.

PROGRESS: As to possible correlations between peripheral endocrine profiles and tissue receptor status, only preliminary results may be reported. An inverse relationship seem to exist between Pri levels (both diurnal and nocturnal) and presence and concentration of E2 receptors, particularly in postmenopausal patients (66.6% RE positive for Pri less than 10 ng/ml versus 37.5% for Pri greater than 10 ng/ml). Histological grading of malignancy allowed us to state that the greater the degree of malignancy allowed us to state that the greater the degree of malignancy the less frequent the presence and concentration of E2 receptors.

118. BIOCHEMICAL ANALYSIS OF HUMAN BREAST CYST FLUID

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OBJECTIVES AND APPROACH: Obtain 150 specimens of breast cyst fluid (BCF) the first year; if distinctive variations are found in biochemical, hormonal, or immunological determinations, then obtain 350 breast cyst fluids the second year and 500 the third year. Analyze these breast cyst fluids for the following components: tumor associated antigens (CEA, TPA, alpha-fetoprotein), chorionic gonadotropin, pituitary gonadotropin, minerals, protein nitrogenous constituents, DNA and polyamines, lipids, enzymes, and hormones. The hormone determinations will be performed by a subcontractor, the Institute for Steroid Research of Montefiore Hospital. Collect all initial clinical information and ongoing clinical follow-up on each patient. Follow-up women who have BCF aspirated and analyzed for at least five (5) years after aspiration to determine if they do or do not develop breast cancer. Provide biostatistical support for computer storage and statistical analysis of the above data.

119. HISTOCHEMICAL METHODS FOR RECEPTORS IN BREAST CANCER

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The summary for this project is under entry no. 90.

120. STUDIES ON URINARY STEROID ANALYSIS IN BREAST CANCER PATIENTS

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OBJECTIVE: To demonstrate steroidal characteristics of a breast cancer patient which are correlated with the initiation and further development of breast neoplasia.

APPROACH: Profile analysis of neutral steroids with gas chromatograph. Urine specimen is processed by Horning's method before GLC analysis.

BIBLIOGRAPHIC REFERENCES: Journal of the National Cancer Institute 59: 49-54, 1977. Hormonal status of breast cancer.

121. MILK PROLACTIN BINDING AND THYROID IN BREAST CANCER

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In lactation, the liver synthesizes most of the carbohydrate and some of the lipid that is secreted in milk, and the liver as well as the mammary glands have receptors for prolactin. We have hypothesized that lactation may be regulated in part by a humoral negative feedback system, involving a factor in milk which diffuses from the breast into the plasma and causes inhibition of prolactin binding in the liver and mammary glands,

thereby decreasing the response of these organs to prolactin. In investigating this hypothesis, we have found that the s.c. administration of bovine milk to female rats significantly decreases the capacity of the liver cell membranes to specifically bind 125I-ovine prolactin. We propose to: a) attempt to identify the prolactin-binding inhibiting factor (PBIF) in milk; b) examine its mechanism of action; c) determine whether it inhibits prolactin-binding in the lactating mammary gland, and in the mammary carcinomas induced by dimethylbenz(a)anthracene (DMBA) and N-nitrosomethylurea (NMU); d) study the effect of PBIF on the growth of DMBA and NMU-induced mammary carcinomas, which are known to be prolactin-dependent; e) determine whether PBIF is produced by these tumors; and f) observe whether it affects the binding of hormones other than prolactin.

There is evidence that NMU produces its carcinogenic effect by methylating the guanine of DNA in the O6 position. In the rat, hypothyroidism decreases the activity of the 'DNA repair enzyme' that removes O6-alkylguanine. We have produced moderate hypothyroidism in the rat prior to administration of a single i.v. dose of NMU, by administration of propylthiouracil (PTU), and continued the PTU for an additional 14 days. Surprisingly, 3-1/2 months later the incidence of mammary carcinoma was significantly decreased in the PTU-treated rats compared to controls. We propose to selectively examine the effect of moderate hypothyroidism and hyperthyroidism on the induction phase and on the growth phase of NMU-induced mammary carcinomas, and to investigate the molecular mechanism of any observed effects on induction.

122. COMPARATIVE STUDY OF HORMONAL LEVELS IN POST-MENOPAUSAL ENDOMETRIAL CANCER PATIENTS AND IN CASES WITH BENIGN CHANGES

Bremond, A. G., Mikaelian, S., Rochet, Y., Hopital Edouard Herriot, Clinique Gynecologique, Pl. d'Arsonval, 69374 Lyon Cedex 2, France

The assessment of hormonal patterns in post-menopausal women after a surgical operation for endometrial cancer will help to define the risk factors for that cancer. Follicle stimulating hormone, luteinizing hormone and prolactin are determined in the peripheral blood of cancer cases and controls, and 5-androstenedione, testosterone, estradiol and progesterone are also measured in the blood of the ovarian vein.

123. ROLE OF ADRENAL CORTEX IN THE ETIOLOGY OF ENDOMETRIAL CANCER

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The high frequency of adrenal hyperplasia in women with endometrial carcinoma is well-known and we have recently demonstrated increased levels of total oestrone as well as of the adrenal androgen dehydroepiandrosterone sulphate in postmenopausal women with endometrial carcinoma. In the proposed project we plan to investigate possible correlation between adrenal production of androgens and peripheral conversion into oestrogens on the one hand and endometrial carcinoma on the other hand.

124. A SEROEPIDEMIOLOGIC STUDY OF ENDOGENOUS SEX HORMONES IN NEOPLASTIC DISEASE OF THE ENDOMETRIUM

Woolever, C. A., Bhavnani, B. R., Clarke, A., McMaster University, School of Medicine, Dept. of Obstetrics & Gynecology, 1200 Main St. W., Hamilton, Ontario, L8S 4J9, Canada

We will determine the serum concentration of sex hormones in three groups of women, i.e., those having a recent diagnosis of carcinoma of the endometrium, hyperplasia of the endometrium and no endometrial disease. Serum samples are obtained from women who have had a recent histologic diagnosis of these conditions, as well as from a control population of age-matched women living in the same neighborhood as the cases. The samples are being analyzed for androstenedione, estrone, estradiol, estriol and their conjugates. Intergroup comparisons will be made with respect to the presence and absence of recent disease and the use of exogenous estrogens. Sample collection will be completed in April 1979 and the analyses by the end of the grant year.

125. ESTROGEN METABOLISM IN PATIENTS WITH ENDOMETRIAL CARCINOMA - EFFECT OF MEDROXYPROGESTERONE ON THE PROGNOSIS OF ENDOMETRIAL CARCINOMA

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The summary for this project is under entry no. 107.

126. BIOCHEMICAL AND CLINICAL ASPECTS OF STEROID RECEPTORS

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The macromolecules in mammalian uterine cytosols responsible for the high-affinity and specific binding of steroid hormones, in particular progestins and glucocorticoids, are the object of this study. Working with purified preparations, we intend to study the interactions of steroid receptor complexes with nuclear material, the relationship of these interactions to the biological effects of the hormones and the possible clinical value of receptor studies in the diagnostic and prognostic assessment of endometrial cancer. The uterine cytosols will be fractionated on spheroidal hydroxyapatite columns to separate the progestin-specific receptor from other high-affinity binding proteins. Endometrial cultures and suppression techniques will be used to study the nuclear interactions of receptor complexes and the hormonal effects. The determination of bound steroids will be carried out by means of a DEAE-cellulose filtration assay.

BIBLIOGRAPHIC REFERENCES: Shapiro, S.S., Dyer, R.D. & Colas, A.E. (1980) Progesterone-induced Glycogen Accumulation in Human Endometrium during Organ Culture, *Amer. J. Obstet. & Gynecol.*, 136, 419-425. Malmoud, I.Y., Klicka, J., Cyrus, R. & Colas, A.E. (1980) The Rate of Conversion of (4-14C)Pregnenolone to (4-14C)Progesterone by Corpora Lutea of the Snapping Turtle, *Chelydra serpentina*. *Gen. Comp. Endocrinol.*, 41, 274-277.

127. DIAGNOSIS AND CONTROL OF PATIENTS WITH TROPHOBLASTIC DISEASE BY THE QUANTIFICATION OF HUMAN CHORIONIC GONADOTROPIN (ABBREV)

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OBJECTIVE: To utilize serial immunological and biological determinations of HCG in patients with trophoblastomas as an auxiliary element in diagnosis and a criterion of cure.

METHODS: The serial quantification by immunobiologic methods is used as an auxiliary reference in the diagnosis of patients who might have trophoblastic disease. In those already treated for the above-mentioned pathology, HCG is titrated (with hypophyseal block) using biological methods (modified Albert).

128. SYNTHESIS, PROCESSING AND SECRETION OF CHORIONIC GONADOTROPIN (HCG) SUBUNITS BY HUMAN MALIGNANT CELLS

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The objectives are to determine the mechanisms controlling the production and release of hCG from human cancer cells. Since a number of studies suggests that the prevalence of hCG-producing tumors in patients is greater than that indicated by the incidence of elevated hCG levels in the plasma (determined by comparing tumor tissue levels and plasma levels), the use of hCG as a tumor marker could potentially be improved if the mechanisms controlling the secretion of hCG subunits by cancer cells were determined. The synthesis, processing and secretion of hCG subunits are being studied in cultured human tumor cell lines, previously shown in our laboratory to be producers of one or both hCG subunits. Pulse and pulse-chase experiments, utilizing immunoprecipitation of radioactively labeled hCG subunits, indicate that high-mannose oligosaccharide-

containing precursors of hCG-alpha and hCG-beta, with half lives greater than or equal to 1 hour, are present in these cells. The intracellular precursors of alpha have apparent molecular weights of 15,000 and 18,000 by SDS-PAGE, and the precursors of beta are 18,000 and 24,000 Mr. Fully processed, mature subunits do not accumulate intracellularly but are secreted into the chase medium 1 to 2 hours (depending on the cell line) after initiation of the chase. These data indicate that there is a rate-limiting step in the processing pathway for hCG subunits and that once the mature forms of the subunits are generated, secretion occurs rapidly. The rate-limiting step appears to be at the point of alpha-mannosidase cleavage of the high-mannose core. Further experiments will examine the carbohydrate composition and amino acid sequence of the intracellular and secreted forms of the hCG subunits produced by malignant cells.

129. ZOLLINGER-ELLISON SYNDROME STUDIES

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PURPOSE: To measure a-HCG (alpha human chorionic gonadotropin) levels in gastrinoma patients and patients with duodenal ulcer disease. Also to evaluate Z-E (gastrinoma) patients, under both stimulated and basal conditions, to determine whether or not prolactin levels can be used as an indicator of the malignancy of these adenomas.

A total of 36 ZES patients have thus far been evaluated with serum prolactin determinations. Eight of these have been found to have elevated levels and seven are thought to have pituitary prolactinomas. Tissue confirmation has been made in 2 and the remaining 5 have suggestive x-ray and/or thyrotropin releasing hormone stimulation tests. These patients are essentially asymptomatic and are being followed with serial prolactin measurements and close clinical observation. These patients, and our other ZES patients, will continue to be followed.

130. RADIOIMMUNOASSAY OF PITUITARY AND CHORIONIC GONADOTROPIN IN ENDOMETRIAL CANCER

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This is part of a broader project. A summary of this subproject is not available.

131. RADIOIODINATED ANDROGENS IN THE DETECTION AND THERAPY OF PROSTATE DISORDERS

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OBJECTIVES: Investigations of the prostate imaging pharmaceuticals. Research on a gamma-bioassay for the assessment of androgen receptors. Labeling of androgens and their antagonists with short-lived radionuclides such as F-18, and the subsequent use of these tracers for the visualization of male sex glands.

APPROACH: (1) Novel synthesis of F-18 labeled compounds via mercury (II) salts; (2) electrochemical labeling utilizing suitable redox couples (inorganic salts) or in an electrochemical cell, and (3) studies related to dextran coated charcoal assay and density gradient centrifugation.

PROGRESS: Papers: J. Label. Compd. 15,7,667; 16,177, Cancer Treatment Reports, 63,1148. A gamma-bioassay for prostatic receptors is ready to be routinely used.

132. THE ROLE OF STEROID RECEPTOR PROTEINS IN DIAGNOSIS AND MANAGEMENT OF BENIGN AND MALIGNANT PROSTATIC DISEASES

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The summary for this project is under entry no. 110.

133. PROSTATE STEROID RECEPTORS BY FLUORESCENCE MICROSCOPY

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The summary for this project is under entry no. 111.

134. STEROID HORMONE RECEPTORS AND CIRCULATING HORMONE LEVELS IN GYNECOLOGICAL MALIGNANCIES

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The summary for this project is under entry no. 108.

135. STUDIES OF SEX STEROID RECEPTORS IN PROSTATIC CARCINOMA TISSUE

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OBJECTIVE: The assessment of the presence and activity of sex steroid receptors in prostatic carcinoma tissue in order to develop a model system to predict the response of patients to hormonal manipulation.

APPROACH: Two assays are under development. a) Cell culture assay: A single cell suspension is obtained by enzymatic dissociation of prostatic carcinoma tissue during prostatic resection or biopsy. The acid phosphatase produced by these cells, in the absence and presence of androgens and estrogens added to the culture medium, is compared. The in vitro behavior of the patients' prostatic carcinoma cells is compared with their in vivo reaction to endocrine manipulation. b) Characterization of cytosol and nuclear androgen receptors: Protamine sulphate precipitates of standard nuclear and cytosol fractions of prostatic tissue are obtained. Binding kinetics and substrate specificities of these receptor fractions are determined, using 3H-dihydrotestosterone (3H-DHT) and 3H-methyltrienolone (3H-R1881) as ligands. The isoelectric focusing patterns of the receptor proteins are determined.

PROGRESS: a) Cell cultures: In cultures from 32 of 65 patients, acid phosphatase levels were higher in the presence of androgens than in their absence. In 24, there was no effect of androgens on this enzyme level, while in 9, androgens were associated with lower enzyme levels than in the control cultures. Similarly, there were 3 patterns of response of these cells to estrogens. In 30 of 36 cultures, estrogens were associated with either no effect or a fall in acid phosphatase levels. However, in 6 incubations, estrogens were associated with higher enzyme levels than in the control incubations. There has been good correlation between these cultures and the in vivo response to endocrine manipulation in 9 patients. c) Androgen receptors: Preliminary studies have shown that the protamine sulphate precipitates of both subcellular fractions bind both 3H-DHT and 3H-R1881 with high affinity. The specificity of these receptors differs from that of serum sex hormone binding globulin (SHBG) in that the affinity for DHT is 10 to the third power the affinity for estradiol. Results of isoelectric focusing reveal that the receptors have different isoelectric points from SHBG.

136. USE OF NB RAT LYMPHOMAS AND LYMPHOMA CELL CULTURES FOR STUDYING HORMONAL DEPENDENCY OF TUMORS AND FOR THE BIOASSAY OF LACTOGENIC HORMONES

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The objectives are (1) to develop a bioassay for human growth hormone (hGH), human prolactin (hPRL) and other lactogens, which is suitable for use in, for instance, clinical diagnosis and investigations, (2) to develop an experimental model for studying the mechanisms underlying the progression of tumors from hormonal dependency to autonomy, (3) to define biochemical processes associated with the mitogenic stimulation of lymphoma cells by lactogens.

PRL of various species has been found to be a powerful mitogen for cells cultured from a transplantable Nb rat lymphoma (Gout, Beer and Noble, Cancer Res. 40, 2433 (1980)). The cultures can detect hormonal concentrations well within the physiological range. Recent studies have further shown that the response of the cells is specific for lactogens, including hGH and hPRL; the stimulatory effects of these hormones in a serum sample can be individually blocked by antibodies to the hor-

mones (Tanaka, Shiu, Gout, Beer, Noble and Friesen. *J. Clin. Endocrin. Metab.*: In Press, Nov. 1980). It is planned to define the optimal conditions for the use of the cultures in the specific bioassay of the hormones in sera and tissues, giving attention to such parameters as the specificity of the blocking antibodies, the stability of the cultured cells with respect to their sensitivity to, and dependency on, the mitogenic hormones for growth. It is planned to use the cultures for screening patients, including those having malignancies related to the pituitary.

Some of the transplantable lymphomas in the Nb rat are highly dependent on exogenous estrogen for growth; cells cultured from these lymphomas have shown a high dependency on PRL for growth in vitro. The estrogen dependent lymphomas can be encouraged to progress from hormonal dependency to autonomy by appropriate adjustment of the hormonal status of the host. Similarly, cultured cells from one of the lymphomas can be induced to progress from dependency on PRL for growth in vitro to a condition of autonomy. The relationship of the progression as observed in vivo to that found in the cultures will be investigated. Preliminary studies indicate the differences in the estrogen dependency of lymphoma lines in the animal can be correlated with differences in the PRL requirements in vitro of cells cultured from the lymphomas. (Text Abridged.)

137. HORMONE PRODUCING NONENDOCRINE TUMORS
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Hormone production by nonendocrine tumors may serve as a marker for early detection. 1) Hormone production by nonendocrine tumors will be measured in peripheral circulation, in affluent and effluent blood obtained at catheterization under fluoroscopy or at surgery in tumor and control tissues. 2) The effects of various methods of therapy on hormone production with progression or regression of the disease will be determined. 3) The effect of certain cancers on hormone secretion by endocrine organs will be studied. 4) The endocrine disturbances which may result as side-effects of different methods of therapy of cancer will be studied. 5) Effect of parenteral hyperalimentation on circulating hormone levels will be evaluated. 6) On detection of hormone producing tumors, the tumors will be extracted directly or cultured in vitro to secrete the hormones. The substances thus obtained are then tested for their physical and chemical properties. Chemical and physical characterization including Sephadex gel- and ion-exchange chromatography will be conducted where practical to compare the molecular size of nonendocrine tumor hormones with native hormones. 7) Localization of hormone production by the tumor will be done by immunohistochemistry. 8) Identify and quantitate the specific hormone receptors in breast and endometrial carcinoma. 9) Investigate the effects of prolactin and other polypeptides and steroid hormones on the growth of breast cancer cells in culture. 10) Study the hormonal changes which might occur with adjuvant chemotherapy in treatment of breast cancer patients and correlate the course of the disease with the hormone profile in these patients.

3. THYROID AND PARATHYROID HORMONES

139. RIA FOR HUMAN THYROGLOBULIN

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The objective is to set up human thyroglobulin (hTg) RIA with two purposes: 1) The control of patients with thyroid carcinoma in whom there is suspicion of metastasis; and 2) To measure radiation damage in patients treated with ¹³¹I for hyperthyroidism in order to predict eventual late hypothyroidism. hTg is obtained locally from human thyroid specimens (autopsy) which is precipitated with ammonium sulphate and purified through Sepharose 4 B. This material will be utilized both as standard and labelled antigen. Tagging of hTg will be performed through the lactoperoxidase method and purified through gel filtration (Sepharose 4 B).

In addition, we will set up a method for the detection of antiTg antibodies.

Once the assay is optimized, we will process serum samples of patients with several thyroid diseases and later on, in the group of patients which we want to study.

139. CARCINOMA OF THE THYROID - SERUM THYROGLOBULIN AS A MARKER FOR METASTASES

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OBJECTIVES: (1) Establishment of a radioimmunoassay (RIA) for human thyroglobulin (Htg) and determination of normal ranges. (2) A follow-up study of approximately 180 patients with thyroid carcinoma - the total number diagnosed in this province between 1955 and 1975. An evaluation of serum thyroglobulin as a marker for recurrent or metastatic cancer of the thyroid will be included in the clinical and laboratory assessment of these patients and also in (3) a prospective study of all newly diagnosed patients with cancer of the thyroid.

PROGRESS: Normal range - 0.25 ng/ml. Results in an initial group of patients are being carefully rechecked and compared with values from another center. Approximately two-thirds of all pathology specimens have been reviewed. More nearly uniform protocols are being worked out with pathologists, surgeons, radiotherapists and other physicians.

140. STUDIES IN THYROID DISEASE

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In the diagnosis of thyroid nodules and goiter, further data have been accumulated on the utility of fine needle aspiration cytology in preoperative diagnosis. This has also been an effective means of diagnosing lymphocytic thyroiditis. In the treatment of thyroid cancer, the serum thyroglobulin levels are being evaluated in correlation with the previously completed study of whole body ¹³¹I scanning. The method promises to be useful provided that patients are allowed to become at least mildly hypothyroid. In the treatment of hyperthyroidism, it was found that both iodide and lithium are effective in controlling hyperthyroidism after the use of relatively low dose ¹³¹I therapy. In the amounts used, iodide produced a greater response than lithium. In the syndrome of inappropriate secretion of thyrotropin (TSH), iodothyronine metabolism was studied in two patients. The metabolic clearance of T4 and T3 was low relative to their circulating hormone levels, but the conversion of T4 to T3 was not impaired. This suggests a possible defect in the entry of thyroid hormones into cells. In the syndrome of postpartum thyroiditis with transient hypothyroidism, several additional patients have been detected and studied.

BIBLIOGRAPHIC REFERENCES: Goldman, J., Line, B., Aamodt, R., and Robbins, J.: Influence of triiodothyronine withdrawal time on ¹³¹I uptake post-thyroidectomy for thyroid cancer. *J. Clin. Endo. Metab.* 50: 734-739, 1980. Robbins, J., Rall, J. E., and Gorden, P.: In Bondy, P. K., and Rosenberg, L. E. (Eds.): *Metabolic Control and Disease*, 8th Edition. W. B. Saunders, Philadelphia, 1980, pp. 1325-1425.

141. PITUITARY AND THYROID FUNCTION IN BREAST CANCER

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The objective of this project is to define the roles played by thyroid hormones and prolactin in the etiology and biology of breast cancer. This goal will be met by studies of nuclear triiodothyronine, cell membrane prolactin, and cytoplasmic estrogen receptors, and mitochondrial alpha-glycerophosphate dehydrogenase in experimental rat mammary carcinomas induced by N-nitrosomethylurea, and human breast cancer tissues. The influence of thyroid function and circulating prolactin levels on the receptor proteins and the thyroid hormone-inducible mitochondrial enzyme will be studied. Clinical investigations will be concerned with the influence of previous drug administration on

tumor receptor levels, particular attention being paid to those known to alter plasma prolactin and estrogen levels.

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142. DIAGNOSIS AND TREATMENT OF FAMILIAL MEDULLARY THYROID CARCINOMA - NEW ASPECTS OF THE DISEASE

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OBJECTIVES: 1. Diagnosis and treatment of familial medullary thyroid carcinoma (MCT). 2. Investigation of the effects on calcium-phosphorus metabolism of the raised S-ICT in patients with sporadic and familial MCT. 3. Investigation of the kinetics (secretion rate, volume of distribution, T 1/2 and renal clearance) of calcitonin in patients with MCT.

APPROACH: 1. The screening program will include determinations of basal and pentagastrin-stimulated serum immunoreactive calcitonin (S-ICT) as a tumor marker for MCT. Blood pressure and 24-hour urinary catecholamine excretion will be determined for diagnosis of possible pheochromocytomas. 2. a. Calcium metabolism is investigated by tracer kinetic methods. b. Bone structure is determined by histomorphometric analysis after tetracycline double labeling. c. Serum concentrations of parathyroid hormone and D-vitamins are determined by radioimmunoassay.

PROGRESS: 1. In one family, 5 patients have formerly been treated for MCT. Further, 6 patients have elevated basal and pentagastrin-stimulated S-ICT and are at present undergoing further evaluation. The screening program will be expanded to include the whole family. 2. b. Preliminary investigations have shown increased bone remodelling with increased numbers of osteoclasts and osteoblasts but reduced activity of these cells. c. Preliminary investigations have shown raised 1,25 dihydroxycholecalciferol concentrations in these patients. 3. This investigation is planned.

143. FAMILIAL INCIDENCE AND MULTIPLE MANIFESTATIONS OF MEDULLARY CARCINOMA OF THE THYROID

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The purpose of this study continues to be to evaluate all family members of patients with diagnosed medullary carcinoma of the thyroid. This evaluation includes a) clinical evaluation, b) serum calcitonin by radioimmunoassay before and after calcium infusion, c) serum parathyroid hormone by radioimmunoassay, d) determination of urinary catecholamines. In any patients in whom abnormalities are found, appropriate therapy will be carried out. In those subjects in whom the studies are normal, repeat studies will be done at 6 to 12 month intervals. In this way, this study should a) lead to early diagnosis and treatment of patients with medullary carcinoma of the thyroid before clinical manifestations are apparent, and b) yield additional information about the incidence, inheritance and natural history of this disease.

During the past year, 5 additional new patients suspected of medullary carcinoma of the thyroid have been evaluated, but the studies were normal in all of these patients. The one patient who was identified as a strong suspect for medullary carcinoma of the thyroid three years ago has been in Europe and is still unavailable for reevaluation. This patient will be more thoroughly investigated and her family members will be evaluated as soon as she returns to this area.

144. DETECTION OF MEDULLARY THYROID CANCER IN FAMILIES

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Hereditary medullary carcinoma of the thyroid gland, a malignant neoplasm which is inherited as an autosomal dominant condition, is reliably detected by radioimmunoassay of calcitonin. The objectives of this study are to improve the methods of early detection of this neoplasm and to establish the optimum protocol for screening relatives of patients with medullary thyroid carcinoma. Measurements of calcitonin in plasma following various stimulating procedures are being performed in members of ten families under investigation here and in members of the additional families of new index cases from this general area. Close clinical and laboratory followup of individuals in such families is being undertaken. Such information is important not only in providing the diagnosis of early disease, but also in establishing prognostic data on both treated and untreated individuals. Genetic linkage and chromosomal studies aimed at even earlier detection of this cancer are being performed on these families and other kindreds at collaborating institutions.

BIBLIOGRAPHIC REFERENCES: Block, Melvin A., Jackson, Charles E. Greenawald, Kenneth A., Yott, Joann B. and Tashjian, Armen H., Jr., Clinical Characteristics Distinguishing Hereditary from Sporadic Medullary Thyroid Cancer: Treatment Implications. *Archives of Surgery*, 115:142-148, Feb., 1980. Jackson, Charles E., Block, Melvin A., Greenawald, Kenneth A. and Tashjian, Armen H., Jr., The Two-Mutational-Event Theory in Medullary Thyroid Cancer, *Am J Human Genetics*, 31:704-710, 1979.

145. MONITORING FAMILIAL MEDULLARY CARCINOMA OF THE THYROID

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The syndrome of medullary carcinoma of the thyroid, pheochromocytoma and parathyroid gland hyperplasia or Multiple Endocrine Neoplasia Syndrome-Type II, is transmitted as an autosomal dominant trait with an approximate 50% chance of occurrence in children of an affected parent. In a large New Haven kindred, comprised of more than 120 members living in a close proximity to New Haven, annual screening of individuals 4 years of age and older has been performed for the past 4 years in the ambulatory facilities of the Adult (General) and Children's Clinical Research Centers, Yale-New Haven Medical Center. To December 1979, pentagastrin provocation, 0.5 mcg/kg and measurement of calcitonin by immunoassay was used as the screening procedure for medullary carcinoma. Positive responses were seen only in individuals previously known to have disease. As of 12/1/79 the procedure was altered to use a combined pentagastrin-calcium infusion based on the above recent data. Screening for parathyroid disease is performed simultaneously with the infusion using total and ionized calcium, alkaline phosphatase and immunoreactive parathormone levels. Urinary catecholamines are obtained on 24-hour collections and assayed in the laboratory of Dr. Lewis Landsberg, Beth Israel Hospital, for pre-symptomatic detection of pheochromocytoma.

Genetic analysis of the kindred performed during the year has succeeded in identifying one of the four main branches of the kindred as almost certainly not at risk based on absence of the disease through 4 generations and normal testing via pentagastrin provocation in more senior members of the branch. It is hoped that further analysis of the family pedigree using the clinical histories and responses to provocation will serve to eliminate other members of the family from active follow-up as 'at risk' for MEN Type II.

146. STUDY OF SERUM CEA AND CALCITONIN IN PATIENTS WITH MEDULLARY CARCINOMA OF THE THYROID

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OBJECTIVES: 1) To determine whether serum CEA is elevated in patients with medullary carcinoma of the thyroid. 2) To evaluate the use of serum CEA in diagnosis, monitoring the results of therapy and early diagnosis of metastases. 3) To establish whether determinations of serum CEA are as useful as determinations of serum calcitonin in diagnosis and monitoring

of patients with MCT. 4) To determine whether serum CEA can be useful in screening for presence of disease in relatives of patients with the familial form of MCT.

APPROACH: 1) and 2) Determination of serum CEA by RIA in patients with histologically verified MCT. 3) Determination of serum calcitonin after calcium or pentagastrin infusions or both. 4) Determination of serum CEA in relatives of patients with familial MCT.

PROGRESS: 1) Serum CEA is raised in patients with MCT and is not elevated in other forms of thyroid carcinoma. 2) Serum CEA appears to be a good tumor marker for MCT. 3) Serum CEA in most cases can be correlated with serum calcitonin levels.

Work is still in progress on 2), 3) and 4) of **APPROACH**.

147. INVESTIGATION OF RAISED IMMUNOREACTIVE CALCITONIN LEVELS IN LEUKEMIA

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OBJECTIVE: To determine whether the raised plasma immunoreactive calcitonin levels found in leukemia are due to ectopic production by leukemic cells, and to see if there is any correlation between plasma calcitonin levels and the clinical status of the patient.

APPROACH: The in vitro production of calcitonin by leukemic cells will be investigated using a radioimmunoassay for calcitonin. If activity is detected, immunoperoxidase and immunofluorescent techniques will be used to determine which cells are producing calcitonin. The intracellular localization of calcitonin will be investigated by assaying subcellular fractions of leukemic cells produced on a Beaufay rotor.

Serial measurements of plasma calcitonin levels in leukemic patients will be undertaken.

148. STUDIES OF CALCIUM AND PHOSPHORUS METABOLISM

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Our main activity involves the study of the hormone calcitonin. We are currently exploring the physico-chemical state of calcitonin in the blood. We have developed a sensitive radioreceptor assay for the hormone, in order to determine which of the heterogeneous serum fractions react with receptor. We have also developed a radioimmunoassay for calcitonin in the urine, and have found it to be of considerable value in the diagnosis of medullary thyroid cancer. We have determined urine calcitonin in normal children ages 2-15 years; children up to age 13 have higher values than adults. Children undergoing a diagnosis of medullary thyroid cancer must be compared by sex and age. In addition, we are utilizing serum and urinary calcitonin as a label for lung cancer and breast cancer.

We have found that large amounts of calcitonin exist in several tissues other than the thyroid gland; the gel filtration of this extrathyroidal calcitonin is identical to the monomer. In exploring its presence in muscle we have found that exercise induces a change in the excretion pattern of urinary calcitonin. In our study of pulmonary calcitonin we have localized calcitonin in the Kultschitzky cell of the lung by immunoperoxidase staining. We are pursuing our studies of muscle and lung calcitonin.

149. HORMONE-PRODUCING TUMORS - IN VIVO AND IN VITRO STUDIES OF GROWTH AND FUNCTION

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This research proposal is designed to study in vitro and in vivo the growth and function of tumors associated with abnormal plasma calcitonin levels. Calcitonin will be measured in blood, tissue culture medium, and tissue specimens by radioimmunoassay and immunohistological procedures in combination with biochemical and immunochemical methods. Measurements will also be made of apparently related hormones such as ACTH and beta endorphin. The in vivo studies will be performed in patients with hypercalcitoninemia and malignancy, including medullary thyroid carcinoma and oat-cell carcinoma of the lung and other neural crest tumors, and in a rat model of

a transplantable medullary thyroid carcinoma. Calcitonin measurements will be made in the basal state and during provocative tests of secretion and during therapeutic regimens. The in vitro studies will be performed with monolayer tissue cultures of calcitonin-producing cells from animal and human tumors. These studies will be designed to investigate the basic biological processes of the cells such as growth and hormone biosynthesis and secretion and to identify factors which regulate these processes. Attempts will be made to establish cell lines of these tumors. The normal counterparts of these malignant cells will also be studied for comparison. These studies will be designed to elucidate the pathogenesis of the hypercalcitoninemia of malignancy. This should result in the further development of clinically useful procedures for the early diagnosis and management of patients with these tumors. This project should thus provide both basic and clinical information about hypercalcitoninemia and cancer.

150. CALCITONIN AS A MARKER FOR CANCER OF THE BREAST

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Using a sensitive and specific radioimmunoassay we have developed for the determination of human calcitonin (HCT) in unconcentrated peripheral blood, we propose to study HCT levels in patients with cancer of the breast. Calcitonin is a hypocalcemic, hypophosphatemic polypeptide hormone secreted by C cells of mammalian thyroid. In 1973, we reported finding increased levels in 35% of cancer patients with spontaneous hypercalcemia. During a study of normocalcemic patients with bronchogenic cancer, it became apparent that over 69% of these patients had calcitonin levels above normal. Was this hypercalcitonemia a response to bone resorption and impending hypercalcemia? Could calcitonin be used as a marker in cancer? Coombes et al. confirmed the high incidence of hypercalcitonemia in bronchogenic cancer and, in addition, showed that 100% of the breast cancer patients with skeletal metastases, studied by them, had high values of blood calcitonin which could be augmented by calcium infusion. In our laboratory a preliminary study of 13 women with metastatic breast cancer in the clinic at the Howard University Hospital showed that 69% had elevated HCT levels. (Many of these patients had undergone prior therapy). We intend to correlate HCT levels with the duration of disease, disease-free period, presence and extent of bone metastases, presence of extra-skeletal metastases, effect of therapy and prognosis. Perhaps HCT may prove to be a marker for breast cancer.

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151. ISOLATION, CHARACTERIZATION AND DEVELOPMENT OF ASSAYS SPECIFIC TO THE MAJOR FORMS OF PEPTIDE HORMONES IN CANCER PATIENTS

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The major objectives are to identify the immunoreactive forms of peptide hormones present in the sera of cancer patients and to study their biosynthesis and secretion in cloned cell lines of human origin. Our data indicate that the major forms of calcitonin in cancer patients' sera exhibit molecular weights greater than or equal to 30,000 and that the calcitonin monomer represents only a minor constituent of the total immunoreactive calcitonin. Our results indicate, however, that certain forms of immunoreactive calcitonin may result from aggregation or binding to other serum proteins. Human cloned cell lines that produce the major forms of calcitonin observed in cancer sera have been developed. Currently, we are isolating these forms of calcitonin from tumors developed in nude mice in order both to

characterize them biochemically and to develop radioimmunoassays specific for their detection.

152. PLASMA CALCITONIN - USE IN EARLY DETECTION AND PROGNOSIS OF CANCER (HUMAN)

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This is part of a broader project. A summary of this subproject is not available.

4. OTHER SPECIFIC CANCER HORMONES

153. ENDOCRINE TUMORS OF THE PANCREAS

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OBJECTIVES: Clinical features of patients with endocrine tumors of the pancreas are extremely variable, depending on the hormone produced by the tumor. Furthermore, our recent observations indicate that these tumors produce multiple hormones. The project seeks to clarify the relation between the clinical features and the hormone produced by the tumor.

APPROACH: Using radioimmunoassay, plasma levels and tumor contents of various hormones, such as insulin, C-peptide, glucagon, pancreatic polypeptide, somatostatin, VIP, gastrin, calcitonin, secretin, motilin, ACTH, beta-MSH and substance P, are being examined.

BIBLIOGRAPHIC REFERENCES: K. Abe, K. Yamaguchi, I. Adachi and N. Yanaihara: Multiple hormone production in islet-cell tumors. In *Proinsulin, Insulin, C-peptide*. Edited by S. Baba, T. Kaneko and N. Yanaihara. Excerpta Medica, Amsterdam-Oxford, 1979, pp. 374-379. K. Yamaguchi, K. Abe, S. Miyakawa, S. Ohnami, I. Adachi, Y. Oka, M. Ueda, T. Kameya and N. Yanaihara: Multiple hormone production in endocrine tumors of the pancreas. In *Out Peptides*. Edited by A. Miyoshi, Kodansha, Tokyo, 1980, (in press).

154. CHARACTERIZATION OF A PANCREATIC ACINAR CARCINOMA

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The overall objectives of this project are to gain a basic understanding of the mechanism of hormone-mediated discharge in the pancreatic acinar cell, and to establish an experimental model for pancreatic cancer to aid in the study of etiology, diagnosis and treatment of this disease. This is being approached by characterizing the hormone-responsiveness and surface membrane components of a moderately differentiated transplantable rat pancreatic acinar carcinoma (J.K. Reddy and M.S. Rao (1977) *Science*, 198: 78-80). Specific aims are to: (1) Compare the discharge response of normal pancreatic acini to that of tumor cells regarding efficacy and potency of secretagogues, increases in cGMP, changes in intracellular calcium, and the role of calmodulin; (2) compare surface properties in terms of number, affinity and distribution of peptide hormone receptors and antigenic or iodinated components on the plasma membrane; and (3) adapt the pancreatic carcinoma to in vitro culture in order to study differentiation and morphogenesis. Normal acini and dissociated tumor cells will be prepared using purified collagenase. Secretion stimulated by cerulein, carbamylcholine, and other agents will be measured by release of amylase and pulse-labeled protein. Antisera to tumor cells will be absorbed with normal tissue and used in immunoprecipitation of detergent-solubilized cell extracts coupled with cell iodination, gel electrophoresis and autoradiography. Characterization will be aided by correlation of results from all areas.

155. HORMONE PRODUCING TUMORS OF PANCREAS (HUMAN)

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This is part of a broader project. A summary of this subproject is not available.

156. RELATIONSHIP OF FECAL NEUTRAL STEROIDS TO LARGE BOWEL CANCER RISK

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The objectives of this study are to analyze excretion patterns of long chain fatty acids and neutral steroids in populations at known risk for colon cancer, to analyze for fecal mutagens in these populations, and to determine if patterns of excretion of fecal neutral steroids or long chain fatty acids correlate with fecal mutagen production. Degradation of cholesterol in the colons of populations at low risk and high risk for colon cancer will be measured and concentrations of oleic acid and neutral steroids in the feces of populations at different risk levels will be compared. Fecal samples from South Africa, Scandinavia and the United States will also be extracted with ether and the extracts tested for mutagenicity using the *Salmonella* mutagenicity test of Ames. To date it has been noted that excretion of mutagens was higher in a population at high risk for colon cancer than in two populations of low risk (Ehrlich et al, *Mutat. Res.* 64, 231-240, 1979). At present it also appears that low fiber diet or genetic predisposition to colon cancer (patients with familial polyposis) did not result in an increased frequency of excretion of fecal mutagens.

157. CLINICAL EVALUATION OF THE HORMONAL RECEPTORS AND THE CEA TISSUE VALUES IN RECTAL CANCER

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OBJECTIVES: A comparison of grading, CEA tissue values and cancer hormonal receptors. 2) A comparative study of grading and histotypes with a double blind evaluation of the pre-operative biopsy and specimen by different pathologists. 3) A comparison of grading, hormonal receptors, CEA tissue values, and T.N.M. classification, local recurrences and survival.

158. INFERIOR PETROSAL SINUS CATHETERIZATION IN DIAGNOSIS AND TREATMENT OF PITUITARY TUMORS

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Inferior petrosal sinus opacification by retrograde venography has been used at several centers as an alternate technique for opacification of orbital veins. In addition, the procedure delineates the anatomy of the cavernous and intrasellar venous sinuses and provides an opportunity for obtaining blood samples directly from the cavernous sinuses. We propose that it may offer definitive methods for the diagnosis of pituitary tumors and that sampling the effluent for hormones will provide evidence for the source of hypersecreted hormones when that source is in doubt. To establish whether this is the case, patients with disorders of the pituitary-adrenal axis who are undergoing continuous venous catheterization for other reasons will have sampling of the inferior petrosal sinus for hormone measurement, and, when indicated, opacification of the cavernous sinus.

159. HORMONES IN CEREBROSPINAL FLUID

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A reevaluation of the significance of pituitary hormones in cerebrospinal fluid has followed the accumulation of a larger series of patients. Most pituitary hormones are present in CSF of normal patients in very low concentrations. Prolactin, for reasons not completely clear, is present in normal patients in relatively higher concentrations so that the ratio of plasma to CSF prolactin concentration is normally about 10:1. In patients with intrasellar pituitary tumors or with the empty-sella syndrome CSF values of all hormones measured (growth hormone, ACTH, TSH, LH, FSH), except prolactin, are at low concentrations similar to those in normals. Prolactin values maintain, however, the same ratio as in normals. In contrast, in patients with suprasellar tumors, CSF values of individual hormones may be very high, suggesting direct release from the tumor into

adjacent CSF spaces. Prolactin also may rise inappropriately (plasma:CSF ratio, 10:1) so that prolactin determinations are of value in assessing suprasellar extension. Additionally, a fall in CSF hormone values after surgical treatment has been found in patients successfully treated for suprasellar tumors. A determination of CSF pituitary hormone values therefore is useful when: 1) Patients are unable or unwilling to have a pneumoencephalogram, and 2) in follow-up of prior treatment for suprasellar extension.

160. MARKER PEPTIDE AND HORMONE PRODUCTION BY ENDOCRINE AND NON-ENDOCRINE TUMORS

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OBJECTIVE: To study the natural history of tumors of the diffuse endocrine system (apudomas) in order to facilitate early diagnosis and (successful) treatment.

APPROACH: Material from endocrine tumors, human and experimental, is subjected to radioimmunoassay, and to cytochemical and immunocytochemical examinations. Antibodies to a wide spectrum of endocrine peptides are applied and the results are correlated, in the case of human tumors, 1) with clinical symptoms and 2) with serum hormone levels.

PROGRESS: An increasingly large proportion of apudomas are found to be producing more than one (hormonal) peptide. In particular, pancreatic polypeptide (PP) has been established as an important marker for pancreatic tumors. Early diagnosis, especially in cases with high circulating levels of VIP, has enabled us to show that micronodular hyperplasia precedes tumor formation. ACTH-secreting apudomas (pituitary and ectopic) have been shown to contain opioid peptides (endorphins) and these may account for unexplained mental effects in Cushing's syndrome.

161. SMALL CELL LUNG CANCER (SCC) AND PEPTIDE HORMONES

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The survival of small cell lung cancer (SCC) patients has recently been improved by more effective chemotherapy. Problems related to the light microscopic classification and recognition of the less well differentiated lung cancers, including SCC, are now more relevant. In addition, new pathologic, kinetic and biochemical alterations have been observed following intensive therapy. This proposal will further explore the relationship of the frequently associated peptide hormone-like product in SCC. Studies in patients, tissue cultures and nude mice with tumor xenografts are designed to increase our understanding of the biology of SCC in relation to the other types of lung cancer. This proposal is designed to provide information that will ensure improved patient management due to better understanding of the clinical, biochemical (metabolic) and pathologic heterogeneity often observed and to developing of an immunocytochemical classification (to aid in the recognition of SCC) and clinically useful biologic markers (to aid in the more accurate assessment of the progression and regression of the neoplasm). Immunoperoxidase methods, radioimmunoassays, standard suspension cultures, soft agar cultures and tumor xenografts in nude mice will be utilized to study the peptides: ACTH, BSMH, BLPH, BEND, AVP and calcitonin. SCC and non-SCC patients will be serially evaluated before therapy for the presence of the peptide in their tumors (immunoperoxidase method) and in their plasma and other body fluids (radioimmunoassays). The in vitro model (tissue cultures) and the in vivo model (xenografts in nude mice) will be serially established to compare the biochemistry in these models with that in the patients and to further characterize peptide content, location, and secretion.

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162. IMPLICATION OF ECTOPIC HORMONE PRODUCTION IN MALIGNANCY

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OBJECTIVE: The principal objective is to explore possibilities of utilizing hormone markers in early detection and diagnosis of cancer. Other aims of the study include elucidation of (1) biological role of specific ectopic hormones produced on non-target tissues of the host. (2) factors involved in the production and release of the same and (3) localization of the actual site of synthesis of such hormones.

APPROACH: Protein hormones are being determined by radioimmunoassay in serum samples of patients bearing different forms of malignant tumors. After the pilot survey, the tumor (biopsy or operated material) tissue from patients with positive hormone assay will be cultured in vitro to isolate and characterize the cell type/types responsible for the hormone production.

The biological role of these hormones is being investigated in experimental animals, employing exogenous hormonal stress. This is done at present with respect to the effects of the hormones on hematopoietic cell proliferation, differentiation and release and on the growth patterns of transplantable tumor.

163. DEVELOPMENT AND USE OF RADIOIMMUNOASSAYS FOR VASOPRESSIN AND NEUROPHYSIN

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Our laboratory has accomplished a satisfactory sensitive radioimmunoassay for vasopressin. The antibody is derived from rabbits immunized with lysine vasopressin conjugated to thyroglobulin. Duplicate samples vary by less than 0.8 pg; recovery of vasopressin added to pooled human plasma is more than 80 percent over the range of 5-20 pg/ml; the assay recognizes lysine vasopressin and arginine vasopressin equally with less than .0005 percent cross-reactivity with arginine vasotocin and oxytocin. In normal human volunteers, the vasopressin plasma level is less than 5 pg/ml in the hydrated state with a plasma osmolality less than 280 mOsm/kg. Above this osmolality in the dehydrated state, vasopressin levels rise rapidly to 10-15 pg/ml.

Studies now in progress are pursuing the possibility that vasopressin is a tumor marker in oat cell carcinoma of the lung and examining the involvement of vasopressin in hypertension with suppressed renin activity. The development of a radioimmunoassay for vasopressin related neurophysin has been deferred in the meantime. Publications: 1) Hamilton BP, Upton GW, Amatruda TT Jr: Peptides with ADH-like activity in tumors producing the syndrome of inappropriate antidiuresis. *Endocrinology* 88, Suppl. A:205, 1971; 2) Hamilton BPM, Upton GV, Amatruda TT Jr: Evidence for presence of neurophysin in tumors producing the syndrome of inappropriate antidiuresis. *J. Clin. Endocrin. Metab.* 35:764, 1972 and 3) Hamilton BP: Presence of neurophysin proteins in tumors associated with the syndrome of inappropriate ADH secretion. *Ann. N.Y. Acad. Sci.* 248:153-156, Feb. 1965.

Current status (Jan. 1980): The radioimmunoassay of vasopressin is being used to study a number of clinical conditions associated with defects in water metabolism. The usefulness of vasopressin as a possible tumor marker in the early diagnosis of cancer is also being explored. The assay of neurophysin has not been found to be useful in the clinical condition study and has been abandoned.

164. STEROID RECEPTORS IN SPECIFIC CELL TYPES IN BPH

Hechter, O. M., Grayhack, J., Scarpelli, D., Lee, C., Bruner Lorand, J., Northwestern University, Chicago Campus, School of Medicine, Dept. of Physiology, 303 E. Chicago Ave., Chicago, Illinois, 60611, United States of America

Collaborative studies will be undertaken at Northwestern University Medical School (Chicago) and Lab Hormones (Biceter, France) to identify the nature and cellular distribution of nuclear steroid receptors in isolated specific cell types of BPH. In Chicago, techniques for isolating viable prostate cells by various methods will be undertaken, including suprafusion of tissue

fragments with appropriate tissue disaggregating enzymes. Stromal and epithelial cells will be separated by gradient fractionation. These cell studies will be correlated with light and electron microscopy. 'Enzyme' markers for cell types and suitable steroid receptor assay conditions will be defined at Bicetre. Optimal methods once developed, will then be routinely applied to BPH samples at both Chicago and Bicetre. The steroid receptor results obtained on isolated prostate cells will be correlated with cell morphology in BPH samples. In Chicago, the nature and distribution of steroid receptor in the cells of 'normal' prostate, both from the peripheral as well as periurthral portions of the gland will also be studied. Finally, nuclear steroid receptors in the specific cell types isolated from prostatic cancer samples will be examined in Chicago.

165. 24-HOUR PLASMA CORTISOL PROFILES TO DISTINGUISH CUSHING'S SYNDROME

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This is part of a broader project. A summary of this subproject is not available.

D. OTHER MARKERS IN CANCER DIAGNOSIS AND PROGNOSIS

166. POTENTIAL ROLE OF THE POLYAMINES IN THE DIAGNOSIS, PROGNOSIS, AND THERAPY OF TUMORS

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Recent studies on the metabolism and function of polyamines have been stimulated by findings suggesting that cellular polyamine levels increase during the early phases of all growth processes. Hence, the particular attention they receive in cancer research.

The most active clinical area of study concerns putrescine, spermidine and spermine changes in tumors. They are found to be high in the serum, plasma and urine of cancer patients; their increase is explained by the spontaneous death and lysis of tumor cells.

Recently it has been observed that in responsive patients, therapeutic treatment, stimulating cell death, produces a temporary increase of polyamines (especially spermidine) in the urine, serum and plasma, followed by a decrease toward normal values. This observation suggests that polyamine changes may be very useful in assessing whether or not the therapy will prove to be valid.

We intend: 1) To investigate the effectiveness of the polyamine test during therapy by comparing the clinical data with the urinary polyamine changes. Data, obtained from the urine of leukemic patients treated by chemotherapy (Tumori, 66,22, 1980), have already confirmed the validity of the test. Studies are under way in urine of patients with other kinds of tumors and other forms of therapy-surgery, radiotherapy. 2) To determine the polyamine levels and the activity of their biosynthetic enzymes in normal and neoplastic tissues in order to confirm that the polyamine alterations in a tissue can serve as a marker of cellular activity. Normal breast tissue and carcinoma breast are at present under investigation.

167. POLYAMINES AS BIOCHEMICAL MARKERS OF MALIGNANT GROWTH

Della Pietra, G., Porta, R., Rogliani, E., Abbruzzese, A., Esposito, C., Camardella, M., Martin, A., Università degli Studi di Napoli, Fac. di Medicina e Chirurgia, Dept. of Biochemistry, via Orazio 31, 80100 Naples, Italy

Our project is to study the usefulness of polyamine determination in biological fluids and in neoplastic tissues in the clinical evaluation of patients with cancer and the modifications of the enzymes of polyamine biosynthesis in malignant growth.

Our data in human larynx carcinomas have demonstrated an increase of polyamines (in particular, putrescine) and of the spermidine/spermine ratio closely related to histological tumor kinetics.

The same research has been extended to mammary gland tumors. At present studies are in progress on the polyamine

levels in the mammary gland (from buffalo and cow) during pregnancy and lactation and in human mammary gland tumors. Some enzymes of polyamine biosynthesis are also under investigation from the same source. Preliminary results indicate: a) dramatic increase of mammary gland polyamines during pregnancy and lactation and great modifications of polyamine biosynthesis enzymes in the same conditions. Arginase from the lactating mammary gland of the cow has been purified and molecular and kinetic properties have been investigated. Our program involves the investigation in the model of lactating and neoplastic mammary gland of the sequence of events (variations of polyamine levels, modifications of enzyme activities, synthesis of peptide-linked polyamines) after hormonal or mutagenic gland stimulation.

168. POLYAMINE RADIOIMMUNOASSAY STUDIES IN CANCER

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Major objectives of this proposal are to provide the biochemist with: 1) Quality antibodies for radioimmunoassay (RIA) exploration of the polyamine (PA) topography of biological fluids; 2) an inexpensive device (RIA) for clinical screening and monitoring; 3) discriminating body fluid PA profiles for pathogenetic studies; 4) antibodies (ABs) for use in experimental biology and pathology.

Employing ABs against spermine (SPM) in a PA RIA we measure picogram quantities of free PA directly in 10-20 microliters of serum. Spermidine (SPD) AB production has been achieved and the RIA has proven highly successful.

Clinical testing, including application of new ABs to RIA, will continue on cancer, normal and disease control groups. Mixed AB single tube RIAs such as anti-SPM plus anti-SPD will be tested for screening efficacy, i.e., high risk groups. Comparison of results of patient sample aliquots using PA-RIAs, mass spectrometry and high pressure liquid chromatography will continue.

Macromolecular carrier conjugate of organo-synthesized T-formation antigens of SPM, SPD, putrescine and other PA congeners including the acetylated PAs will be made and conjugate immunization schedules comparing the efficacy of macrophage harvesting with conventional immunization techniques extended. In addition to these maneuvers to enhance AB specificity, ongoing rapid silica gel column fractionation studies of PAs and affinity chromatography purification of ABs will continue to improve RIA posture in PA biochemistry. PA-RIA picogram sensitivity will allow capillary collection (heel-stick) for serial pediatric blood and spinal fluid examination. Specimen mailers for remote monitor-by-mail will be developed.

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169. POLYAMINES AND HUMAN CANCER

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OBJECTIVE: To develop reliable radioimmunoassays for the determination of polyamines (spermidine, putrescine, and spermine) in plasma and red blood cells of patients with malignant diseases.

The purpose is to assess the value of these assays in monitoring of therapy in oncology.

APPROACH: We chose a sensitive radioimmunoassay for the estimation of the polyamine concentration.

PROGRESS: The estimation by means of the spermidine radioimmunoassay on plasma of patients with multiple myeloma has been proven very valuable and promising.

170. ACETYL POLYAMINES AS MARKERS IN CANCER

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In preliminary studies on a limited number of patients we found that the majority of cancer patients had a higher ratio of N1-acetylsermidine to N8-acetylsermidine and a higher level of monoacetylputrescine in the 24 hour urine than did normal subjects. These findings indicate that the urinary levels of acetyl polyamines may provide a more precise marker for cancer than the urinary levels of total polyamines. The objectives of this proposal are: 1) to establish the nature of the polyamines in urine and plasma of normal subjects and gain a better understanding of the mechanism of renal excretion of the polyamines in humans; 2) to establish the nature of the polyamines in urine of cancer patients and to correlate renal polyamines with progression of cancer and regression after administration of effective chemotherapy; 3) to explore the potential utility of the ratio of N1-acetylsermidine to N8-acetylsermidine and the concentration of acetylputrescine in urine as biochemical markers of cancer and as an early criterion for cancer responding or failing to respond to chemotherapy. The polyamines and acetyl polyamines will be determined by procedures developed in our laboratory which involve high pressure liquid chromatography of the dansyl derivatives of these compounds.

171. FERRITIN VARIANTS IN NORMAL TISSUES, LEUKEMIA AND OTHER MALIGNANT CONDITIONS

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Purification of isoferritins from human acute lymphoblastic leukemia (ALL) cells, chronic granulocytic leukemia (CGL), HeLa cells and normal spleen and myocardium will be followed by their characterization and determination of immunological differences. An attempt will be made to demonstrate differences between ferritin subunits and determine whether ferritin from malignant cells has an abnormal subunit composition. Specific assay procedures will be established where possible for immunologically distinct proteins. Serum collected from patients with acute and chronic leukemia, Hodgkin's disease and breast cancer will be assessed using specific immunoassays to determine the value of these techniques in the detection, follow-up and prognosis of the disease.

172. SERUM FERRITIN LEVEL IN CANCER PATIENTS

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OBJECTIVE: To determine serum levels of ferritin as a tumor marker.

APPROACH: A gel scan method has been evolved for the estimation of serum ferritin levels in cancer patients at the time of diagnosis, during treatment and at the end of therapy.

Ferritin was isolated from sera of normals and patients, by heating at 80 degrees C for 10 minutes in a water bath and extracting with distilled water. It was precipitated by adding ammonium sulphate and kept at 4 degrees C for 12 hours and the precipitate was dissolved in borate buffer. A ferritin solution of 50 micrograms was applied on 7 percent acrylamide gel and the electrophoresis was carried out using borate buffer pH 8.6, 0.05 M. The gels were stained with potassium ferrocyanide solution.

Pure ferritin, obtained from Sigma Chemical Company was used at concentrations ranging from 2-20 for gel electrophoresis. The color densities were determined in a chromoscan-200 and the areas of peaks were used for preparing the standard graph.

PROGRESS: By this technique, the serum ferritin levels of 31 normals and 108 patients with various malignancies have been determined. The normal serum ferritin levels range from 40-108 micrograms/l and for cancer patients the range is 80-8225 micrograms/l. The ferritin levels estimated by this method compare well with those obtained by radioimmunoassay. This technique is elegant, accurate, reproducible and economic.

173. THE CLINICAL SIGNIFICANCE OF FERRITIN AS A TUMOR MARKER

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OBJECTIVE: The goal of the present study is a thorough investigation of the clinical significance of isoferritin as a tumor

marker. It is clear from the literature that elevated levels of serum ferritin are not entirely diagnostic of cancer. However, measurement of the acidic 'onco fetal' isoferritins by means of an ELISA technique and phenotyping with cross-electrophoresis seems to be valuable.

APPROACH: Isoferritins will be determined in two ways: A) Enzyme-linked immunosorbent assay (ELISA) - 1) antigen - liver ferritin (L plus HL); antibody - anti-liver ferritin (a-L plus a-HL); 2) antigen - heart ferritin (H plus HL); antibody - anti-heart ferritin (a-H plus a-HL); 3) antigen - mixture (H plus L plus HL); antibody - anti-mixture (a-L, a-H, a-HL); 4) antigen - HeLa cell ferritin (H); antibody - anti-HeLa cell ferritin (a-H); B) Phenotyping: Isoferritin spectra will be made by means of a cross-electrophoresis procedure. The first dimension is an isoelectric focussing whereas the second dimension will consist of an electro-immuno-diffusion in agar containing antibodies directed against all isoferritins.

PROGRESS: During the last few months we successfully raised highly specific antisera against the distinctive ferritins. The purified ferritins as well as the antisera are going to be used in the ELISA and the cross-electrophoresis.

174. HUMAN ISOERRITINS AND THEIR USE AS BIOLOGICAL MARKERS OF NEOPLASIA

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OBJECTIVE: To characterize human isoferritins from different organs and to evaluate their usefulness as biological markers of neoplasia.

APPROACH: Acidic isoferritins have been reported as possible biological markers of neoplasia; however, technical difficulties in the production of antibodies specific for acidic isoferritins have prevented the clinical application of this assay. The chemical purification and characterization of ferritin and of isoferritins from target organs such as heart, placenta and liver is the first step towards this goal. Antiserum to acidic isoferritins will be produced, followed by absorption with normal liver ferritin. Enzyme immunoassays will be developed for acidic isoferritins. A study of the correlation with diagnosis, stage and other biochemical and immunological markers will be performed. The origin of serum acidic isoferritins will be studied with human tumor cells in culture.

PROGRESS: A longitudinal study of serum ferritin was performed on a group of leukemic and breast cancer patients at various stages of the disease. Using an ELISA assay we found that serum ferritin was a valuable adjunct for the follow-up of breast cancer patients. There was a poor correlation between CEA and ferritin levels. Heart and placental type ferritins were isolated and characterized, and specific antisera were obtained. Ferritin synthesis by lymphoma cells in vitro was found to be phase dependent; also the synthesis of ferritins by these cells in vitro without iron stimulation suggests that the elevated serum levels of ferritin observed and lymphoma in leukemic patients are due to a de novo synthesis.

175. ANALYSIS OF METHYLATED NUCLEOSIDES IN URINE FROM CANCER PATIENTS

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We have set up a method to analyze methylated nucleosides from the urine of cancer patients. We first purify the nucleosides from the urine on a small affinity column and later analyze the content of more than ten different methylated nucleosides using a high performance liquid chromatography system (HPLC). The aim of our investigation is to evaluate whether or not the level of methylated nucleosides in urine from cancer patients may be used as a diagnosis of some tumors.

176. USE OF ALTERATIONS IN THE PATTERN OF MODIFIED NUCLEOSIDES IN SERUM OR URINE IN DETECTION OF CANCER

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The objectives of the research are to establish a sensitive non-invasive diagnostic procedure for the detection of cancer in animal models, and to study the relationship between transfer RNA and cancer. The procedure is based on the findings that

man or animals with neoplasms excrete more than normal amounts of modified nucleosides and bases in their urine. Also, it is planned to determine whether any significant changes occur in the pattern of nucleoside modification in tRNA isolated from Neoplastic tissue as compared to normal tissue.

The experimental animal is the rat and tumors will be induced by specific chemical carcinogens. Urines will be analyzed prior to the development of the neoplasms to determine the time of the earliest changes in the excretion of modified nucleosides. Urines will also be analyzed during the development of the tumors to correlate the excretion of nucleosides with the growth of the neoplasm. At the end of the study, tRNAs isolated from tumor tissue will be compared with those from control tissue to ascertain if, and where, significant differences developed.

177. RADIOHALOGENATED NUCLEOSIDES FOR USE IN DIAGNOSTIC ONCOLOGY

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OBJECTIVE: To design and synthesize radiohalogenated nucleosides which would be of value in allowing a physician to monitor the progress (success or failure) of a therapeutic regimen. These agents are not intended initially to be useful in localizing cancerous tissue, although some may indeed prove useful for lung and breast tumors.

APPROACH: The program involves the synthesis and testing of a number of ¹⁸F- and ⁸²Br-labelled compounds which may be active at the level of DNA synthesis. Specific compounds include 18F-5-F-2'-deoxyuridine (18F-5-FuDR), 18F-2'-F-2'-deoxyuridine (18F-2'-F-2'-UdR) 82Br-2'-Br-2'-deoxyuridine (82Br-2'-Br-2'-UdR) and 82Br-6-Br-uracil. Animal models employed are tumor bearing mice (Lewis lung carcinoma and Ehrlich ascites carcinoma) and rats (Walker 256 and Walker 256 chlorambucil resistant). Testing of the radiohalogenated compounds involves tumor uptake studies, to determine the usefulness of this series of compounds for non-invasive diagnostic procedures in oncology.

PROGRESS: Rapid chemical syntheses of 5-FuDR, 2'-F2'-UdR have been developed. High pressure liquid chromatography (HPLC) is used to purify the products. The synthesis and evaluation of the radiohalogenated 18F and 82Br analogs are in progress.

178. CATECHOLAMINES IN DIAGNOSIS AND THERAPY

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This is part of a broader project. A summary of this subproject is not available.

179. EVALUATION OF BIOMARKERS IN PATIENTS WITH ADVANCED MALIGNANT DISEASE

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Biomarkers when available allow for certain malignancies to be either diagnosed early, recurrences to be diagnosed early, or allow for sequential analysis for the evaluation of treatment. These parameters are usually more sensitive indicators of disease than conventional diagnostic techniques. In an attempt to identify biomarkers from patients with advanced malignancies, blood samples were drawn at the time of admission and sequentially during treatment. Thirty-five patients have had multiple blood samples drawn for CEA, calcitonin, vasopressin, ACTH, and C3dp-C. Clinical correlation with biomarker data will be made to determine if there might be any relationship.

BIBLIOGRAPHIC REFERENCES: Van Echo, D.A., Markus, S., Aisner, J., Wiernik, P.H.: A phase II trial of AMSA (NSC 249992) in patients with metastatic renal cell carcinoma. Cancer Treatment Reports, (in press), 1980. Fuks, J.Z., Van Echo, D.A., Aisner, J., Von Hoff, D., Wiernik, P.H.: A phase II of 4'-(9-acridinylamino)-methanesulfon-m-aniside (AMSA) in patients with refractory small cell carcinoma of the lung (SCCL). Cancer Treatment Reports (in press), 1980.

180. TRACE ELEMENT STUDIES ON CANCER

Ranade, S. S., Shah, S., Chaudhari, C. A., Panday, V. K. Cancer Research Inst., Biophysics Div., Dr. Ernest Borges Marg, c/o Tata Memorial Ctr., Bombay 400012, Maharashtra, India

OBJECTIVE: Trace element profiles are increasingly of interest for differentiating between normal and diseased conditions. The present project is aimed at obtaining trace element composition of tissues in normal and in cancerous states. This is being done with the objective of employing the patterns of some of the trace elements (Fe, Cu, Zn) which could have a diagnostic potential by virtue of their being constituents of metalloenzymes. It is also proposed to evaluate the role of paramagnetic metal ions in magnetic relaxation properties which are known to be altered in malignancy.

APPROACH: Trace element analysis has therefore been undertaken on tissues of normal subjects and of cancer patients by using the technique of atomic absorption spectrophotometry. The elements that are being investigated are Zn, Fe, Co, Mn, Co, Ca, Mg, Na K.

PROGRESS: A significant increase was observed in the levels of Zn, Cu and Mn in the carcinomas of the gastrointestinal tract and leukemic bone marrow compared with the normals. This finding is being confirmed with other cancers for its diagnostic potential.

181. ULTRASENSITIVE ENZYMATIC RADIOIMMUNOASSAY

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By combining radioimmunoassay (RIA) and enzyme-linked immunosorbent assay (ELISA), we have developed a very sensitive immunoassay-ultrasensitive enzymatic radioimmunoassay (USERIA). Experiments were conducted to modify RIA or ELISA so that antigens or antibodies could be measured by the USERIA procedure, and to compare the sensitivity among these immunoassays. So far USERIA has proven to be the most sensitive immunoassay. It is 100- to 1,000-fold more sensitive than RIA in the detection of rotavirus, cytomegalovirus, and cholera toxin. The procedure was modified to determine covalent binding of chemical carcinogens to DNA. The USERIA procedure which measures benzo(a)pyrene (B(a)P)-DNA adducts and acetylaminofluorene (AAF) adducts was 100-fold more sensitive than RIA. As low as 3 fmole AAF DNA adducts in 10 ng DNA can be measured by non-competitive USERIA and 2 fmole in 1 microgram DNA by competitive procedures. With a similar procedure, 4 fmole of B(a)P-DNA adducts in 10 ng DNA can be detected by non-competitive USERIA, and 10 fmole in 25 microgram DNA by competitive methods.

BIBLIOGRAPHIC REFERENCES: Hsu, I.C., Poirier, M., Yuspa, S., Yolken, R., and Harris, C.C.: Ultrasensitive enzymatic radioimmunoassay can detect femtomoles of acetylaminofluorene-DNA adducts. Carcinogenesis, in press, 1980. Hsu, I.C., Yolken, R., and Harris, C.C.: Ultrasensitive Enzymatic Radioimmunoassay. In Methods in Enzymology. New York, Academic Press, in press, 1980.

182. ANEMIA AND PLASMA ERYTHROPOIETIN LEVELS IN CANCER PATIENTS

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OBJECTIVE: Determination of erythropoietin levels in plasma of patients with various cancers and establish a relationship between these parameters in these disorders.

APPROACH: In the proposed study, plasma erythropoietin assays will be rendered more sensitive by the assay of plasma concentrates as we have described recently, Am. J. Med. 66:243, 1979. After written consent is obtained, patients with cancer will be plasmapheresed and approximately 200 ml. of plasma will be concentrated 40 or 60 times. These results will also be related to simultaneous measurements of renal function and arterial blood gases and pH. Hemoglobin and plasma erythropoietin relationship will be determined in these patients.

PROGRESS: Erythropoietin bioassay with polycythemic is being standardized at present time.

183. A SEARCH FOR PRE-NEOPLASTIC CELL MARKERS IN SPUTUM

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The incidence and mortality of lung cancer continue to rise and attempts at 'early' diagnosis to improve prognosis appear to have increased overall five year survivals to no more than 15-20%. We propose that the pathogenesis of lung cancer is a progressive pattern of bronchial epithelial atypias extending over a period of 20-30 years, with first squamous metaplasia followed, in turn, by mild, moderate and severe epithelial atypia and culminating in carcinoma. We believe that by periodic cytologic sputum examination, individuals with moderate to severe epithelial atypias who are at high risk for development of lung cancer can be identified and counseled regarding cessation of smoking, transferred to a less hazardous occupational exposure, and possibly treated with retinoids to prevent further progression of their bronchial atypias. To realize these objectives, we believe it imperative that definite objective morphometric criteria be established for the identification of the atypical bronchial epithelial cells in sputum. To accomplish this goal, we propose to use scanning microscope photometry coupled with various statistical cell image analysis techniques, as were developed in our previous NCI contract (NO1 CP 63837), entitled 'Markers for Evaluation of Pre-Neoplastic Lesions in the Respiratory Tract'.

184. PROSTATIC FLUID IN DIAGNOSIS OF PROSTATIC CANCER

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Analysis of prostatic fluid provides a unique opportunity for studying metabolic changes within the prostate gland. The overall objective of the project is to identify changes in the prostatic fluid composition that are associated with the presence of carcinoma of the prostate. Recognition of the changes has a significant potential to assist in achieving the following goals: 1) Provide an important additional technique for identification of patients with carcinoma of the prostate or those at high risk of developing prostatic cancer. 2) Provide information that may lead to the development of other procedures that will assist in identifying this group of patients. 3) Provide a possible means to identify variations in biological activity of prostatic carcinoma. Our previous studies, utilizing LDH isoenzymes as a biological marker in the prostatic fluid, have demonstrated characteristic ratios of LDH-5/LDH-1 in prostatic fluid specimens from individuals with normal prostate, benign hyperplasia and prostatic cancer. However, prostatic fluid collected from patients with prostatic inflammation (as indicated by the presence of 10 or more white blood cells in the prostatic fluid under the high power microscopic field) showed a wide range of LDH-5/1 ratio in the fluid which overlapped considerably with that of prostatic cancer cases. Our recent attempts at exploring additional markers in the prostatic fluid appeared encouraging. Preliminary data obtained from analyzing C3, C4 complement components and transferrin indicated that elevated levels of these markers were associated with prostatic cancer but not with normal prostate, benign hyperplasia and prostatic inflammation. We propose: 1) To analyze C3 complement and transferrin in conjunction with LDH isoenzymes in the prostatic fluid to confirm our preliminary study in an attempt to increase our ability to identify men with carcinoma of the prostate. 2) To perform a retrospective study to assess the correlation (or lack of it) between the above markers and the stage, grade and biological behavior of the prostatic carcinoma.

185. A SCREENING METHOD FOR MALIGNANCY

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OBJECTIVES: The objective of this project involves evaluating the potential usefulness of plasma levels of protein bound N-acetyl neuraminic acid (NANA) in humans as a potentially useful indicator of malignant disease. Also, the potential

contribution to the elevation of NANA levels by other disease states will be evaluated when possible.

PLAN: Determinations of plasma NANA levels will be done using material from both normal volunteers and patients with known malignancies and other diseases. These determinations will be made using samples which are routinely collected for the determination of carcinoembryonic antigen (CEA) by the Radioimmunoassay (RIA) laboratory. Determinations of both CEA and NANA concentrations will be made using the same sample submitted for evaluation.

METHOD: The Svennerholm technique will be used to determine total NANA. Bound NANA is released by acid hydrolysis, free NANA is then adsorbed by ion exchange chromatography, washed and eluted. A colorimetric procedure is used to determine the NANA concentration present in this sample.

RESULTS: Previous data has shown that the plasma NANA concentration may be a useful clinical indicator for the presence of malignant disease. Results suggests that the normal value for NANA as determined by this technique is 862 micrograms/ml plasma (sigma equals 166), and for all malignancies was 1121 (sigma equals 271).

186. CANCER DETECTION BY SERUM ANALYSIS OF HETEROLYCANES

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This proposal is for support of research to explore a biochemical method of cancer detection based on the quantitation of serum or plasma levels of a lipid-soluble sialic acid (LASA) fraction which is consistently and markedly elevated in tumor-bearing animal models and in human cancer patients. Specific objectives are: 1) To maximize reproducibility and sensitivity of the assay procedure; 2) to correlate LASA levels with tumorigenesis in a broad range of animal models and with several tumor types to gain information on the generality of the procedure as a diagnostic tool; 3) to isolate and identify chemically the chemical constituents of the LASA fraction which are elevated as a result of tumor burden in order to provide basic information necessary to understand the underlying biological mechanisms and to increase specificity and sensitivity of the assay procedure.

BIBLIOGRAPHIC REFERENCES: W.D. Merritt, C.L. Richardson, T.W. Keenan and D.J. Morre. 1978. Gangliosides of liver tumors induced by N-2-fluorenylacetylamide. I. Ganglioside alterations in liver tumorigenesis and normal development. J. Natl. Cancer Inst. 60:1313-1328. W.D. Merritt, D.J. Morre and T.W. Keenan. 1978. Gangliosides of liver tumors induced by N-2-fluorenylacetylamide. II. Alterations in biosynthetic enzymes. J. Natl. Cancer Inst. 60:1329-1337.

187. CYCLIC GMP LEVELS IN GYNECOLOGIC NEOPLASMS

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This proposal is designed to determine the usefulness of cyclic GMP, cyclic AMP assays or their calculated ratios in plasma and urine of patients with gynecological malignancies. As a potential measure of cellular proliferative activity, attention will also be focused upon obtaining data as to whether such values may be useful in the screening, detection, diagnosis and treatment of estrogen dependent neoplasms in the human female.

188. CANCER OF THE HUMAN CERVIX UTERI - EFFICIENCY OF DETERMINATION OF DNA AND LYSINE IN AUTOMATIC GYNECOLOGICAL PRESAMPLES

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OBJECTIVE: During carcinogenesis of the human cervix uteri we have found a significantly higher amount of the basic amino acid Lysine heterochromatin. Therefore the content of Lysine may be a parameter of heterochromatin in the cell nucle-

us, and its efficiency in automatic gynecological prescreening will be evaluated.

APPROACH: Unselected smears were stained and cytophotometrically evaluated. The content of DNA and Lysine was determined and put in histograms. These were compared with cytological and histological findings.

PROGRESS: Preliminary results show that the determination of Lysine and DNA is, in respect to the differential diagnosis (inflammation, hormones, dysplasia, carcinoma in situ and invasive cancer), superior when compared to the determination of DNA only.

189. EARLY BREAST CANCER - THE PREDICTION OF PROGNOSIS

Rubens, R. D., Hayward, J. L., Mills, R., Sexton, S., Egan, M., Bulbrook, R. D., Wang, D. Y., Bell, E., King, R. J., Imperial Cancer Research Fund, Breast Cancer Unit, Lincoln's Inn Fields, London WC2A 3PX, England, United Kingdom

At the present time, the best index of prognosis in early breast cancer is the presence or absence of tumor in axillary nodes. Thus, in order to select high-risk patients for adjuvant therapy, an axillary dissection is necessary. There is a need for other markers of prognosis if radical surgery is to be avoided in every case. Several markers of potential interest for predicting prognosis are available for study. These include plasma and urinary steroids, tumor-enzymes, hydroxyproline excretion, estimations of serum carcinoembryonic antigen, casein, chorionic gonadotropin, methylated-nucleosides or ferritin, tryptophan-metabolite excretion, immunological parameters, and histopathological features. In a prospective study the above are being investigated in patients with early breast cancer. The clinical course of these patients will be correlated with the results of the pre-operative investigations in an attempt to determine factors, or combination of factors, that are of predictive value. (See ICRF Scientific Report 1976 - report 269.)

190. VITAMIN B6 AND RECURRENCE AFTER MASTECTOMY

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Women with breast cancer who excrete small amounts of the principal metabolite of vitamin B6 (4-pyridoxic acid; 4-PA) have a more rapid recurrence rate after mastectomy than those who excrete larger amounts of 4-PA. This may indicate that patients with a poor prognosis are B6 deficient.

A large number of biochemical processes are dependent on an adequate level of B6 and these include endocrine and immune function. Forty-five volunteers have been given B6 (or placebo) and serum immunoglobulins and several plasma hormones are being measured.

Initial results show a significant correlation between the amounts of IgM in the plasma and the excretion of urinary 4-PA after 4 weeks of administration of B6.

It may be of significance that IgM levels in Japanese women with breast cancer are much lower than those in normal Japanese women and are similar to those found in British women (see ICRF Scientific Report 1977 - report 78).

191. GENETIC POLYMORPHISMS IN HIGH AND LOW RISK BREAST CANCER FAMILIES

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The objective of this study is to select a group at high risk of developing breast cancer in the population by means of certain genetic markers. Families with a high incidence of breast cancer have been investigated for a battery of genetic markers, including blood groups, serum groups, HL-A antigens, red cell isoenzymes and chromosome bands. Linkage studies were carried out using the Edwards Computer Program. Studies on plasma prolactin levels in the members of these families were also undertaken. In the first crude analysis of the data, evidence for only a weak linkage between a susceptibility gene for breast cancer and HL-A was found. In order that significant evidence for linkage may be obtained, the number of families must be increased. The material from a study carried out by Dr. H.

Lynch and Dr. Mary C. King is added to the data and analyzed for linkage. The studies of prolactin regulation continue.

192. LABORATORY ASSESSMENT OF PROGNOSIS IN CARCINOMA OF THE BREAST AND LUNG

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OBJECTIVE: Elevated levels of a number of non-specific but malignancy-associated serum factors have been reported in breast and lung cancer patients. Disease-free postoperative stage II breast cancer patients are followed at six week intervals for one year, in an attempt to forecast relapse before it becomes clinically evident since intervention at that juncture might prevent relapse and thus prolong life. In oat cell bronchogenic carcinoma complete remissions are frequently achieved with combination chemotherapy and the completeness of such remissions can be judged by sequential studies of similar serum factors.

APPROACH: Patients with stage II breast cancer or patients with oat cell carcinoma rendered disease free by chemotherapy and radiotherapy are followed at six to eight week intervals with estimations of CEA, ferritin C reactive protein, C1q binding assay for immune complexes.

PROGRESS: In breast cancer, 104 disease-free patients have been followed with the above parameters. Twenty have subsequently relapsed. The serum parameters become abnormal at an average of 6-12 weeks before the relapse becomes clinically evident in 50-80 percent of patients.

193. HYDROXYPROLINE IN BONE METASTASIS FROM BREAST CANCER

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Bone metastasis occurs in 49% of patients with cancer of the breast. Although the diagnosis is currently made by x-rays and scintiscanning, there are no accurate indicators for the evaluation of the early response to therapy. Since bone matrix is the largest store of collagen, the invasion of bone by metastatic cancer is accompanied by an elevated excretion of hydroxyproline (HYPRO) in urine. HYPRO is excreted as dialyzable and non-dialyzable forms; the former represents breakdown of mature collagen, the latter is an index of synthesis of new collagen. It is therefore likely that serial determinations of dialyzable and non-dialyzable HYPRO can be an important tool in the evaluation of therapeutic response in patients with cancer of the breast with skeletal metastasis. We have demonstrated that the post-absorptive HYPRO/creatinine ratio (SPOT-HYPRO in morning urine) is an accurate index of bone involvement in cancer of the breast and multiple myeloma. In continuation of ongoing studies, we plan to determine: (1) the value of SPOT-HYPRO in the screening of patients at risk of developing bone metastasis from breast cancer; (2) if the evaluation of non-dialyzable HYPRO excretion, observed 24-48 hours after chemotherapy is an index of immediate response to therapy and if the test has prognostic value for long-term treatment; (3) if there is correlation between the metastasis/normal bone ratio of densitometry in bone scans with the urinary excretion of HYPRO; (4) the validity of HYPRO measurements as an index of bone metastasis by determining the glucosylgalactosylhydroxylysine/galactosylhydroxylysine ratio in urine. This test differentiates the tissue of origin of the increased collagen turnover as bone or soft tissues.

194. CHARACTERIZATION OF PLASMINOGEN ACTIVATION ACTIVITY IN HUMAN BREAST TUMOR CYTOSOLS

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OBJECTIVE: Plasminogen-activating activities have been demonstrated in human tumors. This study seeks to characterize these activities in human breast tumors.

APPROACH: Human breast tumors being analyzed for steroid receptor proteins will serve as the sources of material. Studies of both cytosol and particulate fractions will be made using recognized separation procedures to characterize the types

and molecular characteristics of the plasminogen-activating activities present in these tumors.

PROGRESS: Plasminogen-activating activities have been demonstrated in breast tumors and have been shown to be weakly correlated with steroid receptor activity. Preliminary studies indicate that further characterization of these activities is possible and suggest that characterization of a tumor's plasminogen-activating activity may provide specific markers for tumor-related functions.

195. SERUM FROM PATIENTS WITH BREAST LESIONS

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OBJECTIVES: Serve as a verification center for new breast cancer specific markers. The intent is to seek innovative approaches in need of large-scale field trials, rather than to add a few more cases to the already well-established studies. Develop specimen resources, i.e., blood from breast cancer patients and from controls, which include apparently normal women, women with benign disease of the breast, women with other types of cancer (gynecological), and women with other diseases.

196. EPR STUDIES ON DETECTION AND TREATMENT OF CANCER

Zimbrick, J. D., Stephens, R., Baker, L., Dean, D., Jewell, W., Mathew, A., University of Kansas, Lawrence Campus, School of Liberal Arts & Sciences, Dept. of Radiation Biophysics, 112 Bailey Hall, Lawrence, Kansas, 66045, United States of America

Electron paramagnetic resonance (EPR) spectroscopy is being evaluated for use as a potential screening technique for early detection of cancer. EPR spin labeling techniques are being utilized to elucidate possible membrane and intracellular viscosity changes which may occur in blood components of humans with cancer. A double-blind study will be continued on the EPR signal intensity of Cu(plus plus) bound to ceruloplasmin (Cu(plus plus)-Cp) and on the ratio Cu(plus plus)/total Cu in serum samples from female volunteers who are asymptomatic but have a higher than average risk of developing breast cancer. Animal tumor model systems already developed will be utilized to provide lymphocytes for spin labeling studies to determine possible changes in membrane fluidity or cytoplasmic viscosity associated with cancer. These tumor models will also be studied to determine how soon after tumor cell implantation the changes in Cu(plus plus)-Cp level and in the ratio Cu(plus plus)/total Cu occur, and to obtain more information on possible hypotheses for these changes. Studies on the molecular properties of CP, including single crystals of CP, will be continued. These will include electron spin echo studies which may help to characterize the binding sites of Cu in the molecule.

BIBLIOGRAPHIC REFERENCES: Horn, R. A., E. J. Friesen, R. L. Stephens, W. R. Hendrick, and J. D. Zimbrick, Electron Spin Resonance Studies on Properties of Ceruloplasmin and transferrin in Blood from Normal Human Subjects and Cancer Patients, *Cancer* 43 (6), 2392-2398 (1979).

197. DETECTION OF HUMAN MILK PROTEIN RNA TRANSCRIPTS IN MAMMARY TUMOR BIOPSIES

Craig, R. K., Davis, M. S., Courtauld Inst. of Biochemistry, London, England, United Kingdom

OBJECTIVE: Development of a nucleic acid hybridization assay for the routine clinical detection of human milk protein RNA transcripts in mammary tumor biopsies.

APPROACH: Isolate and clone, using standard DNA recombinant techniques, mRNA sequences coding for human milk protein. These will then be used to identify the presence or absence of RNA molecules containing milk protein sequences in mammary tumor biopsies.

198. THE DEVELOPMENT OF CDNA PROBES AS DIAGNOSTIC TOOLS IN UNDIFFERENTIATED LEUKEMIA

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OBJECTIVE: To search for differences in the populations of polyadenylated mRNA sequences in cells of different types of human leukemia, and to exploit these differences as markers to distinguish between different types of leukemia, particularly

in those cases for which other markers are poorly delineated or absent.

APPROACH: Polyadenylated mRNA is isolated from cells of patients with well-characterized types of leukemia (AML, ALL, CLL and CGL). Complementary DNA (cDNA) copies of these sequences are synthesized with reverse transcriptase, and homologous and heterologous molecular hybridization reactions are done to measure qualitative and quantitative differences between RNAs from different types of leukemia cells. Heterologous hybridization between the RNA from one type with cDNA transcribed from the RNA of a second type will permit isolation of cDNA probes complementary to RNA sequences which are characteristic (either qualitatively or quantitatively) of the second type of leukemia cell as compared to the first type.

PROGRESS: Some differences between leukemias have been detected, in particular, between CGL and CLL.

199. SERUM AMYLOID A

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During the past year the investigations carried out within this project led to results of some interest in two areas. Firstly, the serum amyloid A level was found to be a close monitor of cancer dissemination and of the response to therapy of patients with metastatic neoplasia. SAA levels above 400 ng/ml were found in 97% of 289 patients with solid tumors with distant metastases, in patients with acute and chronic myelocytic leukemia and patients with advanced lymphoma. They were lower than 400 ng/ml in 250 out of 270 patients with solid malignant tumors with localized and regional disease free of inflammatory processes. Among 20 patients from the latter group with SAA level higher than 400 ng/ml, 16 developed metastases within 214 days from the initial determination. The SAA level decreased in patients who responded to chemotherapy. A statistically significant correlation (p is less than 0.01) was established between the level of SAA and the size of measurable tumors. Secondly, it was established that among various subpopulations of peripheral human leukocytes the polymorphonuclear leukocytes or granulocytes are capable of synthesizing an AA antigenically related material designated granulocyte amyloid A (GAA). It was found that granulocytes, separated from 50 ml heparinized blood from 5 patients with high SAA levels, were able to incorporate ^{14}C leucine (1 microcurie/ml McCoy medium free of leucine) in the de novo synthesized GAA detected in the solubilized granulocyte pellet and in their supernatant with the help of a double radioimmunoassay. The amount of intracellular ^{14}C GAA was found to rise from 155 to 2200 cpm/4 x 10 to the 6th power cells over a period of 20 hours, then to decrease, while the extracellular ^{14}C GAA increased proportionally with the percentage of dead leukocytes. Pyromycin and cycloheximide significantly inhibited the synthesis of ^{14}C GAA, vincristine and colchicine (antitubulin drugs) primarily blocked GAA release from the cells, while hydrocortisone increased its release and endotoxins in small doses increased its rate of synthesis. Preliminary data indicate that GAA is a protein of intermediate molecular weight (approximately 100,000 daltons). (Text Abridged.)

BIBLIOGRAPHIC REFERENCES: Serum Amyloid A: evidence for its origin in polymorphonucleated leukocytes. C.J. Rosenthal and L. Sullivan, *J. Clin. Invest.* 62: 1181-1186, 1978.

200. BIOCHEMICAL CELL MARKER STUDIES IN HUMAN LEUKEMIA AND LYMPHOMA

Strauchen, J. A., U.S. Veterans Admin., Dept. of Medicine & Surgery, Medical Center, Laboratory Service, 3801 Junipero Serra Blvd., Palo Alto, California, 94304, United States of America

OBJECTIVES: Development of immunologic and biochemical markers for the classification of normal and neoplastic lymphoid cells.

RESEARCH PLAN: Current emphasis is on the development of fluoresceinated lectins as probes for the carbohydrate makeup of the cell surface of lymphoid cells and correlation with morphology and enzymatic markers such as terminal transferase.

METHODOLOGY: Lymphocyte isolation and immunologic characterization by immunofluorescence. Lectin binding assay utilizes a panel of fluorescein conjugates and fluorescent phase contrast microscopy.

FINDINGS: Lectin binding to normal lymphocytes establishes major determinants D-Mannose, D Galactose, N-acetyl glucosamine. PHA transformation associated with changes in carbohydrate makeup.

CLINICAL RELATIONSHIP: Development of markers will be useful in diagnosis and classification of lymphoid neoplasms.

201. THE ESTIMATION AND CLINICAL SIGNIFICANCE OF PLASMA AND CELLULAR CONCENTRATIONS OF 6-THIOGUANINE

Maddocks, J. L., Dooley, T., Hallamshire Hospital, Dept. of Clinical Pharmacology, Sheffield S10 2RX, England, United Kingdom

OBJECTIVE: To devise a simple, rapid, specific and sensitive assay for measuring therapeutic concentrations of 6-thioguanine in plasma and cells and to conduct preliminary clinical studies.

APPROACH: To use a highly selective, solvent extraction procedure coupled with fluorimetric detection.

PROGRESS: A specific assay for 6-thioguanine in human plasma has been developed with a sensitivity of 5 ng/ml. An oral dose of 160 mg of thioguanine was administered to a patient with leukemia. A peak plasma concentration of 460 ng/ml occurred at 4 hours and by 6 hours it dropped to 160 ng/ml.

202. TRACE ELEMENT STUDIES ON CANCER

Ranade, S. S., Shah, S., Chaudhari, C. A., Panday, V. K., Cancer Research Inst., Biophysics Div., Dr. Ernest Borges Marg. c/o Tata Memorial Ctr., Bombay 400012, Maharashtra, India

The summary for this project is under entry no. 180.

II. IMMUNOLOGICAL STUDIES IN CANCER DIAGNOSIS AND PROGNOSIS

A. DETECTION AND CHARACTERIZATION OF MONOCLONAL ANTIBODIES

203. USE OF MONOCLONAL ANTIBODIES IN STUDIES ON HUMAN LEUKEMIA

Greaves, M. F., Kemshead, J., Rao, J., Robinson, J., Verbi, W., Imperial Cancer Research Fund, Membrane Immunology Laboratory, Lincoln's Inn Fields, London WC2A 3PX, England, United Kingdom

A panel of eight monoclonal antibodies including anti-HLA-A,B, beta-2 and HLA-D 'framework' or conservative region determinants (provided by the laboratories of Dr. Crumpton and the Director), anti-T (Levy, Stanford; McMichael, Oxford) and anti-brain (Kennett, Pittsburgh) have been screened against normal and leukemic cells and cell lines using the Fluorescence Activated Cell Sorter. Highly selective patterns of reactivity are seen which correspond with previously defined phenotypic subclasses of leukemia. Some of these monoclonals are now used routinely for leukemia immunodiagnosis and subclassification (e.g., DA2/anti-HLA-D, NA134/anti-T) and are also being used to investigate the antigenic phenotype of hemopoietic progenitor cells (e.g., BFUe, CFUe, CFUc).

204. PREPARATION AND EVALUATION OF SPECIFIC MONOCLONAL ANTIBODIES FOR DETAILED ANALYSIS OF THE SURFACE MEMBRANE PHENOTYPES OF LEUKEMIC CELLS

Zola, H., Bradley, J., Brooks, D., Flinders University of South Australia, Flinders Medical Centre, Dept. of Immunology, Bedford Park, S Australia 5042, Australia

OBJECTIVE: The objective of this project is to prepare monoclonal antibodies against lymphocyte membrane determinants which may prove useful in the analysis of leukemic cells. It is already well-known that antibodies against antigens such as the Ia antigen or the antigen characteristic of the common type of acute lymphoblastic leukemia have a useful role in the diagnostic laboratory. It is reasonable to anticipate that further anal-

ysis of membrane phenotype of leukemic cells may reveal more diagnostically useful markers. Furthermore, the analysis of the differentiation antigens found on leukemic cells may help to establish the relationship between different leukemias and different stages of differentiation of normal hematopoietic cells.

APPROACH: The Kohler and Milstein hybridoma technique is being used to prepare monoclonal antibodies against antigens found on normal lymphocytes, lymphoblastoid cell lines, and leukemia cells.

PROGRESS: A number of monoclonal antibodies have been prepared which react with antigens found on different lymphocyte subpopulations. The distribution of the relevant antigens on normal and leukemic cells is at present under investigation. One of the monoclonal antibodies, FMC1 reacts with normal and leukemic B lymphocytes only. Another monoclonal antibody, FMC4 reacts with the human Ia antigen and is useful in the identification of the common (non-T, non-B) type of ALL. FMC3 reacts with a proportion of the T and B cells in normal blood. The leukemic cells from a minority of patients with chronic lymphocytic leukemia react with FMC3 whilst the cells from the majority do not.

Further monoclonal antibodies against different specificities on normal and leukemic cells are being prepared and evaluated.

BIBLIOGRAPHIC REFERENCES: Brooks, D.A., Beckman, I., Bradley, J., McNamara, P.J., Thomas, Miriam E. and Zola, H. (1979). Human lymphocyte markers defined by antibodies derived from somatic cell hybrids. I. A hybridoma secreting antibody against a marker specific for human B lymphocytes. Clin. Exp. Immunology.

205. HUMAN HYBRIDOMA ANTIBODIES IN NEOPLASTIC DISEASE

Kaplan, H. S., Olsson, L., Stanford University, School of Medicine, Dept. of Radiology, 300 Pasteur Dr., Stanford, California, 94305, United States of America

We propose to use methods recently developed in this laboratory to generate monoclonal human-human hybridoma antibodies against the cell membrane antigens which define the phenotypes of different types of human neoplasms, with particular emphasis on the lymphomas and leukemias. In vitro assays will be used to select antibodies with high binding affinities and/or cytotoxicity for later use in clinical diagnosis and treatment of the corresponding neoplasms. After checking for monoclonality, sterility, and freedom from pyrogens, these antibodies will be tested over an increasing dose range in Phase I trials in patients with advanced neoplastic disease to establish acceptable dose levels. They will also be labeled with ¹²⁵I or other suitable radionuclides and injected into Phase I patients to ascertain whether selective uptake can be reliably detected in primary neoplasms, regional lymph node metastases, and distant metastatic sites. These antibodies will be used in direct or indirect immunofluorescence and immunoperoxidase studies to assess their usefulness in the differential diagnosis of various types of lymphomas and in selected other types of neoplasms. If such Phase I studies yield encouraging results, randomized clinical trials will be performed to evaluate the efficacy of cytotoxic or radiolabeled monoclonal human antibodies in the adjuvant treatment of micrometastases in patients with lymphomas and leukemias, and subsequently in patients with other types of neoplastic disease, in whom complete remissions have been induced by conventional treatment. Preliminary consideration will also be given to molecular cloning of cDNA prepared from the mRNA of hybridomas producing selected, high-priority antibodies.

206. CLINICAL APPLICATIONS OF MONOCLONAL ANTIBODIES IN LUNG AND COLON CANCER

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PROJECT OBJECTIVES: 1. Establishment of lymphocyte-myeloma hybrid cell lines producing monoclonal antibodies to cell surface antigens present on human lung cancer. 2. Characterization of the various antigenic determinants expressed on these human tumor cells using monoclonal antibodies as the defining reagents. 3. Evaluation of these monoclonal antibodies as diagnostic reagents. a. In vitro serologic analysis of human tumor antigens and antibodies. b. In vivo imaging of metastatic tumor deposits using radiolabeled monoclonal antibody and con-

ventional scanning procedures. 4. Preliminary evaluation of the therapeutic potential of monoclonal antibodies.

PLAN: Paired tumor cells and autologous lymphoblastoid cells will be used to identify antigenic determinants preferentially expressed on the tumor. The tissue distribution and specificity of these antigens will then be evaluated. Monoclonal antibodies showing specificity will be used to develop immunodiagnostic assays.

METHOD: Tissue culture, somatic cell hybridization, radioimmunoassay, enzyme-linked immunoassay, column chromatography, and gel electrophoresis are the techniques which will be employed.

CLINICAL RELATIONSHIP: This area of research is relevant to the earlier diagnosis of both the primary tumor and recurrent disease in lung and colon patients.

207. IMMUNODIAGNOSIS OF MALIGNANT LYMPHOMAS IN TISSUE SECTIONS WITH PURIFIED ANTIBODIES

Janossy, G., Thomas, J. A., University of London, Royal Free Hospital School of Medicine, Dept. of Immunology, 8 Hunter St., London WC1N 1BP, England, United Kingdom

OBJECTIVES AND APPROACH: Purified antibodies are prepared which specifically react with the following human antigens: kappa and lambda chains, IgM, IgG, and IgA immunoglobulin classes, T lymphocyte/thymocyte antigen, B cell associated Ia-like (p28,33) antigens and monocyte/myeloid antigen. Pure antibodies (produced by mouse B cell-myeloma hybrids) are selected which react with (sub-)populations of human T and B lymphoid cells and monocytes. These reagents are used on tissue sections in different combinations using double fluorescence assays. The aims of the study are: (a) To distinguish malignant (monoclonal) lymphomas from benign (polyclonal) lymphoid hyperplasias in cases which pose diagnostic problems and (b) to analyze subset heterogeneity of T-B lymphoid populations in normal and malignant lymphoid tissues, and to analyze the usefulness of recently developed monoclonal antibodies (sensitized by mouse B cell myeloma hybrids) for lymphoma diagnosis.

B. DETECTION AND CHARACTERIZATION OF OTHER ANTI-TUMOR ANTIBODIES

208. PATTERNS OF EXPRESSION OF LYMPHOCYTE SURFACE IMMUNOGLOBULINS IN CHRONIC LYMPHOCYTIC LEUKEMIA

Ling, N. R., Dhaliwal, H. S., University of Birmingham, Dept. of Immunology, P.O. Box 363, Birmingham, England, United Kingdom

OBJECTIVE: To attempt to correlate surface immunoglobulins in lymphoproliferative disease with other diagnostic features and with prognosis.

APPROACH: Testing of large numbers of CLL with a panel of surface marker tests, and particularly with a new and especially sensitive rosette test for SmIg. Gathering of general clinical information following course of disease.

PROGRESS: Large amount of data. Technically successful, but data yet to be analyzed. Some success in early diagnosis of B cell lymphoproliferative diseases.

209. CORRELATION OF MORPHOLOGICAL CHARACTERISTICS OF NON-HODGKIN'S LYMPHOMAS AND LEUKEMIAS WITH SURFACE IMMUNOGLOBULIN

Sukumaran, M. S., Majhi, U., Rajalakshmi, K. R., Cancer Inst., Dept. of Pathology, Adyar, Madras 600020, Madras, India

The study of cellular characteristics of tumors has acquired great significance for determining the prognosis of non-Hodgkin's lymphomas and acute lymphatic leukemias. There have been many attempts to classify these tumors, based on morphological, functional, or immunological characteristics, into T and B cell lymphomas and leukemias. None of these methods appears to be specific when used singly. The clinical behavior and the prognosis of the condition depends on the cell type. We intend to study the cellular characteristics by combining morphological, cytochemical and immunological methods, and to type these into B cell and T cell lymphomas or leukemias and correlate these findings with the clinical behavior.

The study of surface immunoglobulin is done by immunofluorescence technique using FITC labelled anti-IgG and IgM antisera. The tumor cell suspension is stained by direct method.

Impression smear from lymph nodes and peripheral blood smears are used for cytochemical study of PAS and acid phosphatase stain. Morphological study is done on imprints stained by Pap smear and also on histological sections. The study has just commenced.

210. NATURAL AND ANTIBODY-DEPENDENT CYTOTOXIC ACTIVITY OF HUMAN LEUKEMIA CELLS

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OBJECTIVE: The natural (SLMC) and antibody-dependent (ADCC) cytotoxic activity is the property of lymphoid cells belonging to 'K' or 'O' populations respectively.

Our investigations cover: the determination of SLMC in acute leukemias; the detection of antibodies or factors in the serum that may block or increase the SLMC and ADCC activity; and the correlation between SLMC and ADCC and the clinical state of the patients.

APPROACH: The SLMC and ADCC activity is measured by chromium-release technique using K-562 cell line and chicken red blood cell targets. Separation of T, B, and 'O' cells is also employed to detect the cytotoxic activity of subsets.

PROGRESS: It is hoped that results of these studies would render data of diagnostic value, which may aid in monitoring the effects of therapy.

211. IN VIVO LOCALIZATION OF LABELLED TUMOR-SPECIFIC ANTIBODY FOR DIAGNOSTIC SCANNING AND FOR THERAPY

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OBJECTIVE: To study the interaction of anti-tumor antibody with tumor cells and endeavor to obtain a degree of localization in animal tumors suitable for the purpose of carrying scanning agents or the tumor.

APPROACH: Antibody to L5178Y mouse lymphoma has been prepared in a goat with partially purified tumor antigen. This was obtained by precipitating from 3M KCL extract of tumor cells those antigens which correspond to the antibodies in an anti-normal spleen cell serum. IgG was prepared by affinity chromatography. The time course of injected 125 I-labelled antibody in the tumor, blood and organs of mice was observed. The inactivation of antibody by cells has been studied in vitro. Antibodies released by tumor cells under normal culture conditions have, to a large extent, lost the ability to bind to tumor cells. Degradation of antibody is evident from fractionations on Sephadex G-200. It is intended to produce large quantities of tumor-specific high affinity antibody by fusion of antibody producing spleen cells from an immunized mouse with a myeloma cell line. 211 AT and 125 I will be used as agents to be coupled to antibody.

PROGRESS: A number of different immunosorbents have been tested for the purification of antibody to L5178Y mouse lymphoma. For an IgG preparation with an antibody content of 45% the tumor to blood concentration ratio was about 1 compared to an expected ratio of about 10. A rapid removal of antibody (compared to normal IgG from the tumor-blood system has been observed. This indicates that factors besides the purity of antibody limits the localization of antibodies in tumors. Antibody released from tumor cells in vitro has been shown (on filtration to Sephadex G-200) to contain antigen-antibody complexes and enzymatic degradation of IgG. Experiments in progress are aimed at the production of hybrid cells of antibody producing B cells from an immunized mouse and/or myeloma cell line.

212. IMMUNOLOGICAL RETARDATION AND PROGNOSTICATION OF OSTEOSARCOMA

Cambin, J. G., Enneking, W. F., Spanier, S., U.S. Veterans Admin., Dept. of Medicine & Surgery, Medical Center, Gainesville, Florida, 32601, United States of America

Evidence has shown that osteosarcomas, like their animal counterparts, carry tumor specific cell surface antigens which

can induce an antibody response and an immunological reaction in the tumor bearing patient. Preliminary experimental results using serum obtained from osteosarcoma patients, patients with other related tumors and also normal controls have shown that there is an antibody response present in the osteosarcoma patients as shown by hemagglutination studies in those patients who have been exposed to osteosarcoma or have had the disease and are currently clinically well. This titer has fallen in those patients in which the disease is active or has progressed. In parallel with these results a test for cellular responses as opposed to humoral using a lymphocyte adhesive inhibition tests has shown that those patients with active disease have marked loss of lymphocyte adhesiveness in the presence of tumor antigen whereas those patients who are clinically well show almost a 90 percent adhesive response when challenged with the osteosarcoma antigen. A test batch of assorted sera have been sent to Dr. Hugh Fudenberg of Charleston S.C., who as an immunologist has assayed these samples and found that there is a specific type of antibody present in those patients with the osteosarcoma and have confirmed the results as demonstrated by the more crude passive hemagglutination assay.

A pilot study using Dunn osteosarcoma and C3H/HeJ mice has shown that the tumor grows easily, forms an osteoid and bone and will metastasize to the lungs as does the human variety. By means of tumor antigen vaccination of these mice and by injection of sensitized leukocytes obtained from C57 black mice, tumor growth in the C3H mice was retarded. Numbers of mice in the study were small and it is projected that a larger study may show a statistically significant response. However, the response in these few mice did compare favorably with those obtained in 28 human subjects using cross-implants and leukocyte transfer. Now there is no increase in mortality and 40 percent of patients are still disease-free 5 years from initial treatment. By using more purified antigenic material we hope that the study will prove more accurate in predicting how well patients will do.

213. ANTISERA TO HUMAN NEUROBLASTOMA CELLS

Kemshad, J., Bicknell, D., Greaves, M., Imperial Cancer Research Fund, Membrane Immunology Laboratory, Lincoln's Inn Fields, London WC2A 3PX, England, United Kingdom

We are attempting to produce antisera that will unequivocally identify small numbers of neuroblastoma cells infiltrating bone marrow of patients.

Studies on antisera raised to several neuroblastoma cell lines, either in rabbits or by somatic cell hybridization studies, suggest a marked heterogeneity of cell surface antigens on different lines. Examination of the binding of neurotoxins (bungarotoxin, tetanus) and cholera toxin also suggests substantial variation in the expression of surface binding sites for these ligands. These observations have further been confirmed by study of bone marrow samples, known by histological examination to be heavily infiltrated with neuroblasts. Whilst with certain antisera some samples showed good reactivity, with others no binding could be demonstrated.

The marked heterogeneity of surface antigenic expression within the tumors defined as 'neuroblastoma' may be explained by the observation that neuroblastoma is capable of spontaneous or induced differentiation/maturation to a benign tumor (ganglioneuroma). Different antigenic expression may correspond to the arrest of cells at different maturation stages. In vitro studies on the differentiation of human neuroblastoma lines are in progress to test this possibility.

Hybridoma (monoclonal) antibody-defined surface antigens so far tested are shared with at least one hemopoietic lineage (e.g., granulocytes or B cells). Differentiation-linked cell surface antigens on hemopoietic cells, T cell antigens, HLA-D/la' antigens are not detectable on neuroblastoma cells.

214. BREAST NEOPLASIA DIAGNOSIS WITH SPECIFIC ANTIBODIES

Ceriani, R. L., Childrens Hospital Medical Center of Northern California, Bruce Lyon Memorial Research Laboratory, 51st & Grove Sts., Oakland, California, 94609, United States of America

Antibodies against cell-type specific antigens on the surface of mammary epithelial cells (both in humans and in mice) are used for the development of diagnostic procedures for the early and specific detection of breast cancer. These mammary epithe-

lial antigens are shown, by indirect immunofluorescence, to be present on breast cells both in vivo and in vitro, irrespective of their origins (normal or neoplastic breast tissue). They are not detected on cells from other tissues. By quantitative techniques that we have developed to measure these antigens on a cell per cell basis using the flow cytofluorometer, we have shown that neoplastic breast epithelial cells contain levels of mammary epithelial antigens per unit DNA several fold less than levels in normal breast cells. We have also developed very sensitive radioimmunoassay techniques, with which we have established that mammary epithelial antigens are released into circulation of mammary tumor-bearing mice, but not in normal mice. This finding sets the basis for the development of mass screening methods for early detection of breast cancer, since the radioimmunoassay is a simple, inexpensive and highly sensitive procedure. Also, methods are being developed for the localization of breast cancer metastases by whole body scintiscanning techniques using radioactively labeled antibodies against the breast epithelial antigens.

BIBLIOGRAPHIC REFERENCES: Wilbanks, T., Peterson, J. A., Miller, S., Kaufman, L., Ortendahl, D. and Ceriani, R. L. Imaging of Mammary Tumors with Cell-type Specific Antibodies with a High Purity Germanium Camera. Presented at Radioimmunochemical Detection of Cancer: A Symposium, Albuquerque, New Mexico, December 1979, in press. Fentiman, I. S., Hurst, J., Ceriani, R. L., Taylor-Papadimitriou, J. Junctional Intercellular Communication Pattern of Cultured Human Breast Cancer Cells. *Cancer Research* 39:4739-4743, 1979.

215. THE LOCALIZATION OF METASTATIC ADENOCARCINOMA OF THE PROSTATE BY RADIOLABELLED PROSTATIC ACID PHOSPHATASE ANTIBODIES

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OBJECTIVE: The feasibility of localizing human prostatic tumors using radiolabelled antibody against prostatic acid phosphatase is being investigated.

APPROACH: A model system employing athymic 'nude' mice as hosts for human prostatic acid phosphatase producing tumors is being developed. The in vivo tissue distribution of xenogenic prostatic acid phosphatase antibody will be studied in tumor bearing animals. If tissue specificity for human prostatic tumors in the model system can be shown, clinical trials in patients with adenocarcinoma of the prostate will be carried out. It is hoped that early lymphatic metastases to the regional lymph nodes will be shown. This would permit more accurate staging of prostatic cancer and allow more effective and rational treatment.

216. IMMUNE COMPLEXES AND SPECIFIC ANTISERA IN OVARIAN CANCER PATIENTS - A PROGNOSTIC INDEX AND THERAPY MONITOR (ABBREV)

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OBJECTIVE: To evaluate whether immunoassay of circulating tumor-associated products in ovarian and other gynecological cancers can be used for diagnostic purposes in monitoring tumor burden and assessing responses to therapy.

APPROACH: Screening tests of Clq precipitation for immune complexes using serum and ascites fluid. Immunotherapy trial comparing two treatment groups: 1. Surgery plus chemotherapy. 2. Surgery plus chemotherapy plus BCG immunotherapy.

PROGRESS: No elevation of immune complexes has been found to be associated with ovarian cancer using direct Clq, indirect Clq, indirect heat inactivated or Raji assays. Levels have been found to be raised in other gynecological tumors, e.g., carcinoma of the cervix, vulva and body of the uterus. The BCG immunotherapy trial is in progress.

Fourteen patients have been treated on a double-blind basis with BCG or placebo. Results so far do not indicate any alteration of prognosis with intraperitoneal BCG.

**217. EXTERNAL DETECTION OF TUMORS BY
RADIONUCLIDE LABELED IMMUNOGLOBULINS**

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To date approximately 150 patients (V.A.) have been examined by scanning with antibodies to carcinoembryonic antigen (CEA), alpha-fetal protein (AFP) and human chorionic gonadotropin (HCG) labeled with I-131. Overall sensitivity of the method is about 80% for tumor detection. This figure could be higher except that the resolution limit of available scanning instrumentation is 1-2 cm. Approximately 40 patients with colorectal cancer, 20 with lung cancer, 20 with other digestive tract (pancreas, stomach, esophagus, etc.) cancer have been studied. Plasma levels of circulating antigen do not affect localization by scanning. Surgical specimens have consistently demonstrated tumor to non-tumor ratios of radioactivity to be 2.0-2.5.

The Tc-99m labeling of IgG has been developed to the point of clinical evaluation in lymphoscintigraphic procedures for the detection of nodal metastases. Currently used iodination procedures have been examined as well as the suitability of commercial radioiodide from various suppliers. Amersham and Union Carbide provide products suitable for antibody labeling. The chloramine-T procedure was found to provide the most efficacious labeled proteins in regard to reliability, retention of immunological activity, and suitability for patient administration. Presently studies are underway to evaluate In-111 labeled antibodies. Initial results indicate that anti-CEA IgG can be labeled with In-111 using DTPA as a chelating agent or bridging intermediate.

**218. BRONCHOALVEOLAR LAVAGE FLUID IN
PULMONARY CARCINOMA - SECRETORY
COMPONENT OF IMMUNOGLOBULIN A AS A
MARKER OF NEOPLASTIC GROWTH**

Reynolds, H. Y., Merrill, W. W., D'Esopo, N., U.S. Veterans Admin., Dept. of Medicine & Surgery, Medical Center, Pulmonary Section, W. Spring St., West Haven, Connecticut, 06516, United States of America

OBJECTIVES: To assess the quantity of secretory component (SC) in lower lung airway secretions as an indicator of ciliated epithelial dysfunction in patients at high risk to develop lung cancer (cigarette smokers) or in those who have already developed a lung mass by chest radiograph.

RESEARCH PLAN AND METHODS: The SC glycoprotein molecule is synthesized and secreted by the epithelial cell and it couples subsequently with secretory IgA immunoglobulin. Since there is no effective cure for lung cancer even with optimal surgical extirpation, emphasis must be on early detection of cellular biochemical changes in susceptible people (i.e. epithelial secretion in cigarette smokers) before cytological atypia develops. Currently, lung secretions are obtained by lung lavage (BAL) during diagnostic fiberoptic bronchoscopy in patients with a potentially resectable lung mass. Our protocol is unchanged. The study is being conducted at the West Haven VA and Yale New Haven Hospitals.

RESULTS: In the past year, 17 patients have participated in the study, 7 were hospitalized at the West Haven VA. Since the initiation of this study (1977), a total of 109 patients have participated; of these 42 were patients at the West Haven VA. No significant complications have occurred in these subjects. Results show (1) heavy cigarette smokers have lower SC values (calculated as % of total BAL protein) than light smokers (less than 10 pk-yr) or non-smokers; (2) smoker patients with histologically proven squamous cell carcinoma have very low, often undetectable, SC values and (3) patients with other tumors (adeno-carcinoma or oat cell) have intermediate SC values that probably reflect their smoking history. It appears that the degree of smoking history and the presence of a squamous cell tumor will correlate with diminished SC suggesting a secretory dysfunction of lung epithelium.

**219. THOMSEN-FRIEDENREICH (T) ANTIGEN AND
ANTIBODY IN DIAGNOSIS OF HUMAN PLEURO-
PULMONARY CANCERS**

Springer, G. F., Evanston Hospital, Dept. of Surgery, 2650 Ridge Ave., Evanston, Illinois, 60201, United States of America

No summary has been provided to the Smithsonian Science Information Exchange.

**220. LOCALIZATION OF TUMOR SITES WITH I-131
LABELED ANTIBODIES**

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This is part of a broader project. A summary of this subproject is not available.

**C. DETECTION AND CHARACTERIZATION
OF IMMUNE COMPLEXES**

**221. DETERMINATION OF CIRCULATING IMMUNE
COMPLEXES (CIC) IN SERA OF TUMOR PATIENTS -
DIAGNOSTIC & PROGNOSTIC VALUE &
METHODOLOGICAL EVALUATION**

Casali, P., Barocci, S., Celada, F., Manca, F., Pistoia, V., Sittia, R., Università degli Studi di Genova, Cattedra di Immunologia, Viale Benedetto XV, 10, 16132 Genoa, Italy

OBJECTIVES: Objectives of this project are: a) to compare methods used for the detection of CIC that utilize different 'recognition units'; b) to correlate the 'quality' of CIC with diagnosis and prognosis of cancer patients; c) to analyze complexes isolated from patients' sera to determine isotype of the antibody moiety and nature of antigen.

APPROACH: Development of a competitive method for CIC detection, based on the use of a complex of antibody and the enzyme B-galactosidase. This complex ('probe complex') retains the enzymatic activity and is used as a competitor to measure the binding of test CIC to a variety of 'recognition units' (e.g., conglutinin, C1q, antibody).

PROGRESS: 1) During 1980 we perfected our probe complex method with conglutinin and set up the C1q system. 2) As a cooperative effort, the Italian workshop for Immune Complex has been organized. Ten laboratories in Italy are engaged in this Workshop; a total of one hundred sera are exchanged and tested by seven different methods. A first round has been completed; the results will be discussed after the second round, which is currently in progress. 3) By our Conglutinin-Probe Complex method, a total of 647 cancer sera have been examined. The test yielded a positivity of 24.4%. The types of neoplastic disease with the highest rate were: nasopharyngeal carcinoma, EBV positive (45%), and digestive tract tumors (40%).

**222. ANTIGEN-ANTIBODY COMPLEXES IN CANCER
PATIENTS' SERA**

Rassen, R. D., Baylor College of Medicine, School of Medicine, Dept. of Microbiology & Immunology, 1200 Moursund Ave., Houston, Texas, 77025, United States of America

Radiolabeled tumor cell extracts will be used in quantitative immunoprecipitation assays to estimate the amount of tumor cell-associated antigens recognized by antibodies in unblocked sera from normal donors and from patients with malignant melanoma and other malignancies. The immunoprecipitates will be analyzed by polyacrylamide gel electrophoresis (PAGE) followed by radioautography to determine the number and molecular size of the antigens recognized by these sera. The proteins identified by these methods will be compared with those identified by isoelectric focusing followed by PAGE of the original tumor cell extracts. In this way, we will be able to identify molecular characteristics of distinctive tumor cell surface antigens which are recognized by human alloantisera as a preliminary to their isolation for use in diagnostic radioimmunoassays.

BIBLIOGRAPHIC REFERENCES: Rossen, Roger D. Immunological Considerations in the Glomerulopathies Associated with Neoplastic Tumor. In: The Kidney in Systemic Disease. W. N. Suki and G. Eknoyan (Eds.), John Wiley and Sons, Inc. 1980, in press. Rossen, R.D. and Morgan, A.C., Jr., Blockade of the Humoral Immune Response: Immune Complexes in Cancer. The Handbook of Cancer Immunology, ed. H. Waters, Ph.D., Garland, STPM Press, New York, 1980, in press.

**223. DETECTION AND ANALYSIS OF CIRCULATING
IMMUNE COMPLEXES**

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Health, National Cancer Inst., Dermatology Branch, 9000 Rockville Pike, Bethesda, Maryland, 20205, United States of America

Increasing evidence indicates that circulating antigen-antibody complexes play a role in the pathogenesis of a variety of dermatologic, rheumatologic, neoplastic and infectious disease states. We have identified and partially characterized the immune complexes which exist in several diseases i.e. Sjogren's syndrome, mixed cryoglobulinemia, mixed connective tissue disease, acute and chronic schistosomiasis, hepatitis and various types of cutaneous and systemic vasculitis, utilizing two highly sensitive radioimmunoassays for detecting immune complexes i.e. ¹²⁵I-Clq binding assay and the Raji cell radioimmunoassay. We have developed a new, sensitive radioimmunoassay for the detection of IgA containing immune complexes. We have determined the antibody classes present in the immune complexes and examined the physicochemical characteristics of these complexes, as well as the reaction of these complexes with mediators of inflammation such as the complement system. We have examined the correlation between absolute levels of circulating immune complexes, the extent and severity of clinical disease, and reticuloendothelial system function. We have also examined prospectively and retrospectively the effect of various treatment modalities on serum levels of immune complexes and the correlation with clinical improvement.

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224. STUDY OF CIRCULATING IMMUNE COMPLEXES IN CANCER

Masson, P. L., Cambiaso, C. L., Université Catholique de Louvain, Unit of Experimental Medicine, 75 Ave. Hippocrate, B1200 Brussels, Belgium

OBJECTIVE: To assay and isolate antigen-antibody complexes in the circulation of cancer patients, and to isolate, identify and characterize constituents of the complexes.

APPROACH: The antigen-antibody complexes are detected by inhibition of the agglutinating activity of rheumatoid factor or C'9 IgG-coated particles. The agglutination is measured by counting the non-agglutinated particles in a Technicon Autocounter designed to count blood cells. The method is tested by reference to the results obtained by sucrose gradient ultracentrifugation, which is also used for the isolation of complexes.

PROGRESS: The agglutination inhibition test is now operational and routinely used at a sampling rate of 40/hr. Preliminary data obtained in lung and breast cancer indicate that the level of circulating immune complexes correlates to some extent with tumor burden.

225. CIRCULATING IMMUNE COMPLEXES AND RETICULOENDOTHELIAL FUNCTION IN BREAST CANCER PATIENTS

Bugat, R., Pourrat, J. P., Boneu, A., Centre Claudius Regaud, 11 Rue Piquemil, 31032 Toulouse, France

OBJECTIVE: To study function of the spleen in disposal of circulating immune complexes in cancer patients.

APPROACH: Antigen-antibody complexes have been implicated as agents which block cellular immune responses in the tumor bearing host and some authors presented evidence that the measurement of circulating immune complexes in cancer patients may help in determining prognosis. Considering that the spleen is a major site of uptake of Ag-Ab complexes, we decided to look for any relationship between the function of the reticuloendothelial system and determination of circulating immune complexes in breast cancer patients. We use the clearance of heat-damaged labeled autologous red cells as an overall guide to splenic function; circulating complexes are detected by a Clq-binding assay. Sequential determinations are repeated for each patient during the course of the disease.

226. ANTIGEN-ANTIBODY COMPLEXES IN BREAST CANCER

Chu, T. M., Papsidero, L. D., Nemoto, T., Roswell Park Memorial Inst., 666 Elm St., Buffalo, New York, 14263, United States of America

The objective of this study is to assay soluble antigen-antibody complexes in serum of patients with breast cancer using Clq binding radioimmunoassay, Raji cell radioimmunoassay, and (rabbit) rheumatoid factor assay. The serum specimens will be obtained from breast cancer patients, fibrocystic breast disease patients and breast cancer families as well as from appropriate healthy control individuals. The breast cancer patients will be longitudinally followed for evaluation of the response to treatment in order to determine the prognostic and monitoring values of the assay. The high risk groups (fibrocystic breast disease and breast cancer families) are also to be followed in order to evaluate the diagnostic potential of the immune complex assay. The antigen-antibody complexes are also to be isolated from the serum and body fluids (pleural effusion) of breast cancer patients by molecular exclusion chromatography, protein A affinity chromatography and immunoadsorption using affinity media to the Fc domain of IgG. Further, the isolated immune complexes will be dissociated under the least denaturing conditions. The dissociated antigens are to be characterized biochemically and immunologically. Xenobiotic antisera will be raised with the dissociated antigen(s) and with isolated complexes as well as with the Raji cells containing surface-bound immune complexes injected into rabbits made tolerant to human IgG, and studied by their ability to recombine the dissociated antigen and to bind malignant breast tissues. In vitro antibody-dependent cell-mediated cytotoxicity, lymphocyte mitogenesis, PHA-induced lymphocyte blastogenesis and LAI assays are also to be performed to study the possible biological function of antibody-antigen complexes. Further, a radioimmunoassay will be developed which may be of use in early detection and monitoring of efficacy of therapy for breast cancer.

227. DETECTION OF ANTIGEN-ANTIBODY COMPLEXES IN BREAST CANCER

Medof, M. E., University of Chicago, Pritzker School of Medicine, Dept. of Medicine, 5801 S. Ellis Ave., Chicago, Illinois, 60637, United States of America

OBJECTIVES: Assay a statistically adequate number of sera from patients with breast cancer, benign breast disease, and normal volunteers for IC's by employing multiple techniques for IC detection and quantitation which have been developed in and are currently in operation in our laboratory. Our multiple techniques will permit differentiation between soluble and insoluble IC's and between complement bearing and IgG-Fc accessible IC's. To correlate presence or absence, or levels where applicable, of individual IC subpopulations with tumor progression, response to therapeutic intervention, and clinical course, otherwise. Data will be analyzed according to clinical stage of the patient, histologic type of the tumor, histology of regional lymph nodes, ovarian and other hormonal status of the patient, and the presence or absence of estrogen receptors on tumor cells.

228. STUDY OF IMMUNE COMPLEXES IN PATIENTS WITH LEUKEMIA

Hollan, S. R., Fust, G., Mod, A., National Inst. of Hematology & Blood Transfusion, Dept. of Immunochimistry, Daroczi Ut 24, P.O.B. 44, H1502 Budapest, Hungary

OBJECTIVE: Study of the correlation between the concentration of circulating immune complexes (IC's) and the course of the disease in patients with leukemia. Isolation of the circulating IC's from the sera of patients, characterization of the antigen and antibody components of the complexes. Study of the IC's deposited on the surface of bone marrow and peripheral blood lymphoid cells of patients and clarification of whether the antigen and antibody components of the circulating and deposited IC's are the same.

APPROACH: Measurement of the level of circulating IC's in sera of patients with different types of leukemia by different tests (Clq binding test, Clq solubility test, complement consumption test) in parallel, in several serum samples of the same patients obtained in different stages - blastosis, remission, infectious and bleeding complications of the disease. Investigation of

the effect of chemotherapy and plasmaphoresis on the IC level. Isolation of the IC's from the patients' sera by polyethylene glycol precipitation and/or adsorption chromatography on Clq-Sepharose 4B column. Separation of the antigen and antibody components of the IC's by treatment with buffer of low pH and gel filtration. Study of the ability of the separated antibody fractions to bind onto leukemic blast cells and normal peripheral blood lymphocytes. Measurement by immunodiffusion and/or passive haemagglutination of the capacity of these fractions to react with some postulated antigen part of the circulating IC's (viral antigens, leukemia-associated antigens) or the ability of the separated antigen fractions to react with antisera raised against these antigens. Study of the IC's deposited on the surface of bone marrow and peripheral blood cells of the patients by immunofluorescence, using FITC-labeled anti-Ig and anti-C3 sera as well as (after characterization of the antigen component of the circulating IC's) antisera raised against this (or these) antigen(s).

PROGRESS: Clarification of the possible role of IC's as blocking factors for the cell-mediated immune response against leukemia-associated neoantigens. Evaluation of the effect of plasmaphoresis as a possible approach for the therapy of leukemic patients. Prognostic value of the detection of circulating IC's in leukemia.

229. STUDY OF IMMUNE COMPLEXES IN PATIENTS WITH LYMPHOMAS

Jamra, M., Dorhac, P., Universidade de Sao Paulo, Faculdade de Medicina, Dept. of Hematologia, Ave. Dr. Arnaldo 455, Sao Paulo 01246, Brazil

OBJECTIVES: To study immune complexes in patients with lymphomas, their prognostic value and their relationship with immune status of the patient. The aim of this study is to correlate the level of circulating (non-specific) immune complexes with survival, response to therapy, and immunologic status of the patient as evaluated by skin tests and percentages of circulating B and T lymphocytes. The patients will be subjected to this study prior to and during chemotherapy.

APPROACH: Circulating immune complexes will be sought using the method of radioprecipitation by I31I labeled Clq. Skin tests will be performed applying at least 5 antigens: DNCB, PPD, Trichophyton, Dermatophytin and Varidase. T lymphocytes will be evaluated by the conventional sheep red blood cell rosetting and B lymphocytes by C3 zymosan complexes.

230. CIRCULATING IMMUNE COMPLEX LEVELS IN PATIENTS WITH CANCER OF THE REPRODUCTIVE TRACT

Symonds, E. M., Baldwin, R. W., Clarke, A. G., Faratian, B., University of Nottingham, Cancer Research Campaign Lab., Dept. of Obstetrics & Gynecology, University Park, c/o City Hospital, Nottingham NG7 2RD, England, United Kingdom

OBJECTIVE: To investigate whether circulating immune complexes (CIC) in patients with various gynecological tumors and premalignant disorders can be used to monitor tumor growth, detect early cancers, monitor cytotoxic therapy and enable recognition of recurrence.

APPROACH: Since it is clear that there is no single method of assaying for CIC in patients with gynecological tumors, we propose to use new assay systems on the sera of new and previous patients. These include a comparative study of Clq precipitation with and without the addition of heparin, Con A conglutinin assay and polyethylene-glycol precipitin assay.

PROGRESS: The Clq precipitation assay with and without the addition of heparin has shown that CIC in the sera of pre-operative ovarian patients, of patients with cervix dysplasia, and of patients with carcinoma of the vulva and body of the uterus are not elevated.

In the presence of heparin, however, in sera of patients with cervical carcinoma in situ and invasive cervical carcinoma and in post-operative ovarian sera, CIC are elevated. We have evidence to suggest that heparin may enhance the sensitivity of the binding CIC detected in sera without heparin.

231. IMMUNOLOGICAL MONITORING OF PATIENTS BEARING CEREBRAL ANAPLASTIC GLIOMAS

Guidetti, B., Di Lorenzo, N., Palma, L., Lauro, M. G., Università degli Studi di Roma, Istituto di Neurochirurgia, Viale dell'Università 30, 00185 Rome, Italy

OBJECTIVE: Detection and evaluation of Clq-binding immune complex in the serum from patients carrying brain gliomas in relation to the presence of a humoral immune response, detected by immunofluorescence tests by light microscopy and by immunoperoxidase by electron microscopy, to antigenic determinants occurring in human glial tumoral cells in vitro.

APPROACH: Sera from patients previously screened with immunohistochemical techniques (immunofluorescence and PAP) have been used to detect prospective immune complexes according to the radioimmunological method of the 1125-Clq-Ba described by Zubler et al. (1976). The results thus obtained show the occurrence of immune complexes to an extent increasing with the tumor's malignancy; on the other hand, the same sera tested with immunohistochemical techniques detect antibodies to determinants on the surface of human glial tumoral cells in vitro only in patients carrying highly malignant glial tumors.

232. HUMAN TUMOR ANTIGENS - IMMUNE COMPLEXES AND CANCER

Gold, P., Shuster, J., McGill Cancer Research Centre, 3653 Drummond St., McIntyre Bldg. Room 714, Montreal, Quebec, H3G 1Y6, Canada

The tumor-bearing host develops a specific humoral antitumor immune response. Recently, new technology has demonstrated the presence of immune complexes in the circulation of cancer patients. The nature of these circulating complexes is unknown. Theoretically, these immune complexes should consist of tumor antigen and antitumor antibody. Our objective is to isolate such complexes, and analyze their individual components with the view to purifying the relevant tumor antigen. Paraformaldehyde fixed Raji-cells can be used to selectively bind and then elute the immune complexes present in cancer patients' sera. These complexes are presently being characterized.

To date, at least one constituent has been demonstrated in the sera of cancer patients which may well have tumor antigenic activity.

D. DIAGNOSTIC AND PROGNOSTIC APPLICATIONS OF CEA

233. DEVELOPMENT OF AN IMPROVED METHOD FOR MEASUREMENT OF CARCINOEMBRYONIC ANTIGEN (CEA) BASED ON IMMUNOCHEMILUMINESCENCE

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Clinical studies employing radioimmunoassays for the measurement of CEA have clearly shown that the serum levels of the tumor antigens are related to the diagnosis of colorectal cancers. Moreover they may be of importance in the monitoring of treatment and management of cancer patients. The significance of CEA immunoassays in the evaluation of gastrointestinal cancers is complicated by the molecular heterogeneity of the CEA, as well as by antigenic cross reactivity with components of normal tissue.

This project is undertaken with the aim of increasing the specificity of CEA assays as well as the development of a convenient method of CEA measurement bypassing problems of short life of the reagents. Attempts to increase assay specificity will be based on: a) Purification and preparation of standard CEA from human colon carcinoma cell lines. b) Studies on the immunochemical sites of the CEA molecule employing the standard CEA and trials to isolate fragments of the CEA molecule endowed with high specificity. An enzyme linked immunoassay will be developed utilizing the CEA standards of higher specificity and the chemiluminescent method.

Efficiency of the improved CEA assay will be evaluated by comparison with currently employed radioimmunoassays in clinical trials testing cancer patient sera.

234. ASSAY AND STRUCTURE OF CARCINOEMBRYONIC ANTIGENS

Todd, C. W., Shively, J. E., Clark, B. R., Punde, H., Gibson, D., City of Hope Medical Center, Division of Immunology, 1500 E. Duarte Rd., Duarte, California, 91010, United States of America

The primary objective of this research has been to perform structural studies on carcinoembryonic antigen (CEA) and CEA-related antigens (TEX, NCA) in order to develop more specific immunoassays for the early detection of cancer and monitoring the progress of various types of cancer. Emphasis in the coming year will focus on microsequencing of peptide fragments from CEA and TEX formed by specific cleavages at methionine, tryptophan, lysine, and arginine peptide bonds. NH₂-terminal sequence information will be used to construct DNA probes which will serve as primers for enriching the specific synthesis of cDNA corresponding to CEA mRNA from various cell lines, and as screening agents for the isolation of recombinant DNA containing CEA cDNA. Work on the improvement of CEA immunoassays will focus on the use of monoclonal reagents in enzyme linked immunoassays (ELISA). A pilot study on the comparative use of CEA and TEX radioimmunoassays for serially monitoring cancer patients was recently completed. Expansion of these studies to a larger trial in breast cancer and head and neck cancer is under consideration.

235. HUMAN TUMOR ANTIGENS - STUDIES OF CARCINOEMBRYONIC ANTIGEN

Gold, P., Krantz, M., McGill Cancer Research Centre, 3655 Drummond St., McIntyre Bldg. Room 714, Montreal, Quebec, H3G 1Y6, Canada

OBJECTIVE: Elucidation of the biochemical and immunological relationships between CEA and cross-reacting substances from normal and neoplastic tissues. Information obtained will be used to evaluate the potential for diagnostic assays of improved specificity.

APPROACH: Cross-reacting substances will be purified and compared with CEA by limited proteolysis. Individual fragments will be further characterized by peptide mapping and immunological analysis. Unique fragments will be used to raise antisera with more restricted specificity and clinical investigations will determine if these materials are of use as diagnostic and prognostic tools.

PROGRESS: A large number of CEA fragments have been produced by limited proteolysis and partial characterization has been obtained for 12 of the digestion products. The determination of comparable substances from other tumors and normal tissues should provide a great deal of data of both theoretical and practical interest.

The structure of the CEA molecule has been elucidated electronmicroscopically.

236. IMMUNOBIOLOGY IN GASTRIC CANCER

Janssen, C. W., Tonder, O., Matre, R., Thunold, S., Orjaseter, H., Haukeland Sykehus, Dept. of Surgery, N5016 Bergen, Norway

OBJECTIVE: Immunobiological charting of gastric cancer with particular emphasis on the prognostic significance of immunological parameters.

APPROACH: A) Clinical: Staging, mode of operation in radical procedures, palliative procedures, follow-up. B) Pathological: Staging and grading (S. Thunold). C) Carcinoembryonic antigen preoperatively and during follow-up (H. Orjaseter). D) Immunoglobulins and complements, T and B lymphocytes and their reactivity, markers for mononuclear cells in tumor tissue (O. Tonder and R. Matre).

Relationship of (C) and (D) to prognosis, staging and grading.

PROGRESS: A preliminary report on carcinoembryonic antigen (CEA) according to staging and prognosis has been made (Norw. Surg. Ass. October 78). CEA is unfit as a screening test for gastric cancer. Elevated CEA preoperatively in Stage C is a bad prognostic sign. About 2/3 of the patients in Stage D have elevated CEA. Patients with recurrence after radical operations have increasing CEA, compared to preoperative values.

237. RADIOIMMUNOASSAY OF CEA AND AFP AS A SCREENING TEST IN THE SURVEY OF HEALTH OF WORKERS IN THE CHEMICAL INDUSTRY

Ivanescu, M. A., Mihaela, B., Marie, P., Alexandru, T., Ministry of Chemical Industry, Labour Protection & Hygiene Centre, Radioimmunoassay Laboratory, 39 Pop de Basesti, 73244 Bucharest 3, Romania

Selected groups of workers with exposure to vinyl chloride, benzene, chromium, amino compounds, and black carbon have been examined since 1975 by radioimmunoassay techniques. The results are correlated with numerous laboratory and clinical data in order to evaluate the possibility of using RIA of serum or urine CEA and AFP as a screening test in the survey of health of groups of workers with occupational cancer risk.

238. ESTABLISHMENT OF A METHODOLOGY FOR RADIOIMMUNOASSAY IN CANCER - BASIC PARAMETERS AND NORMAL VALUES OF CARCINOEMBRYONIC ANTIGEN AND AFP

Del Rosario, B., Guzman, C., Solis, J., Abuid, J., Instituto Peruano de Energia Nuclear, Division Aplicaciones, Av. Luis Aldana No. 120, La Victoria Apartado 1687, Lima, Peru

In preliminary report to IAEA on this project, we detailed the results on AFP and CEA determinations by radioimmunoassay in 65 normal subjects of both sexes.

The mean values and its standard deviation were 6.15 plus or minus 2.90 ng/ml. and 6.47 plus or minus 4.75 ng/ml for AFP and CEA respectively. Most subjects were adults, 91% between 21-50 years old. No significant difference related to sex was found and values in some younger patients were of the same magnitude as that in adults.

In the second part of this project sera are being collected from patients with neoplastic and non-neoplastic diseases for investigation of CEA and AFP levels. The neoplastic group will consist of colon and rectal cancer cases and testicular tumors. Beta-subunit of chorionic gonadotropin determinations will be added. Assays to be performed before and after surgery and at intervals, in order to evaluate the reliability of this method as a guide of the evolution and therapy response.

239. PREPARATION OF AN ORIGINAL RIA METHOD FOR DETERMINING CEA OF ADENOCARCINOMA OF THE COLON IN PLASMA

Buraggi, G. L., Bombardieri, E., Righini, R., Rodari, A., Turrin, A., Istituto Nazionale per lo Studio e la Cura dei Tumori, 1 Via G. Venezian, 20133 Milan, Italy

This study intends to set up a CEA radioimmunoassay using glycoproteins isolated from the primitive colon tumors. In fact the use of extracts from hepatic metastases limits the specificity of the methods available at present. We performed several extractions from the primitive tumor to obtain different pools of glycoproteins, as membrane glycoproteins and cytosol glycoproteins.

The biochemical and immunological characterization of these substances allowed us to choose a fraction from cytosol. These CEA-like glycoproteins were extracted by means of perchloric acid; afterwards ConA chromatography and purification by gel filtration were carried out.

In the near future radioimmunological methods will be used to test patients with colorectal carcinomas. Statistical methods will control the diagnostic specificity of this assay as compared to commercial methods.

240. CLINICAL CEA-TUMOR RADIOIMMUNODETECTION

Goldenberg, D. M., Kim, E. E., Nelson, M. O., Choy, Y., Bennett, S., Pettit, W., DeLand, F. H., University of Kentucky, School of Medicine, Dept. of Radiation Medicine/Pathology, Limestone & Euclid, Lexington, Kentucky, 40506, United States of America

The overall objective of the current Grant, CA 25584-01 RAD, was to determine whether a radioiodinated antibody to carcinoembryonic antigen (CEA) could detect and localize tumors by external photoscanning. Based upon our findings in more than 200 patients, we have concluded that his method of 'radioimmunodetection' is a successful and most promising new technology for in vivo tumor radiolocalization.

1. Both primary and secondary epithelial tumors of diverse histopathology could be localized successfully by external scintillation imaging after administration of the appropriate radiola-

beled antibody preparation. Radioiodinated normal goat IgG does not yield comparable results. 2. Such tumor demonstrations correlate very well with other diagnostic findings, and occasionally detected tumors not demonstrable by other techniques. 3. Tumors not known to contain appropriate antigen (CEA or AFP) do not show localization of abnormal radioactivity. 4. Very high titers of circulating antigen do not appear to prevent tumor radiolocalization by the radiolabeled antibody to this antigen. 5. In the majority of studies, the subtraction technology developed in this study is essential for accurate localization of tumors. 6. At the present time, the resolution of the method appears to be 2 cm-diameter tumors. The purpose of the proposed supplement is to confirm and extend these findings at this and at a minimum of five other medical centers. The main objective is to determine the clinical feasibility and utility of this new method of cancer radioimmunodetection, using our own prepared radiolabeled anti-CEA IgG, in a number of specific cancer types and stages.

241. CORRELATION OF CEA CONCENTRATION IN THE SERUM AND IN TUMOR TISSUES WITH ITS HISTOCHEMICAL DEMONSTRABILITY IN TISSUE SECTIONS (ABBREV)

Bagshawe, K. D., Paradinas, F. J., Charing Cross Hospital, Dept. of Medical Oncology & Histopathology, Fulham Palace Rd., London, W6 8RF, England, United Kingdom

OBJECTIVE: To define precisely the localization of carcinoembryonic antigen (CEA) and other tumor markers in surgical specimens of cancers of lung, breast, stomach, colon and rectum and to correlate this information with estimations of the concentration of the same antigens measured by radioimmunoassay on serum obtained preoperatively and by extraction from fragments of the same surgical specimens.

APPROACH: There is evidence from *in vivo* antibody localization studies that CEA may be more widely distributed in tumors than is suggested by elevated serum levels of this marker. Confirmation of this is important if the use of cell membrane markers for antibody targeting is to be widely applicable. Information on the distribution of CEA in various tumor types and on the concentration of CEA in tumor specimens in relation to serum concentration is limited. Frozen and conventionally fixed specimens will be used for tumor marker localization by immunofluorescence and immunoperoxidase techniques. Cells in suspension will be studied and morphometric studies on the tissue preparations performed with carcinoembryonic antigen measured in serum and tissue sections by double antibody radioimmunoassay.

PROGRESS: Project started in the summer of 1979.

242. IDENTIFICATION OF CARCINOEMBRYONIC ANTIGEN IN CYTOLOGY SPECIMENS

Pascal, R. R., Fenoglio, C. M., Columbia University, St. Lukes - Roosevelt Hospital Center, Dept. of Pathology, Amsterdam Ave. & 114th St., New York, New York, 10025, United States of America

The objective of this study has been to evaluate a method for the detection of the tumor-associated antigen, CEA (carcinoembryonic antigen), as an adjunct to standard cytologic examinations of body fluids in cancer screening. The goal has been to use the CEA-immunoperoxidase technique to re-examine body fluids containing 'suspicious' and 'atypical' cells for a more accurate marker for malignancy.

The three-year goal was to examine approximately 5,300 specimens collected routinely in our cytology laboratories. During the past 10 months 1,346 immunohistologic preparations were stained for CEA with appropriate non-immune serum controls. The results are as follows (source, followed by number of CEA-positive preparations, number of CEA-negative preparations, and number of negative controls): pleural - 11, 169, 180; bronchial - 19, 141, 160; sputum - 24, 191, 215; peritoneal - 8, 82, 90; urine - 22, 6, 28.

Among the negative controls (non-immune serum substituted for anti-CEA) only granulocytes and histiocytes containing native peroxidase showed a positive reaction and these were readily distinguished from epithelial cells.

We are currently beginning to review the cytologic diagnoses and clinical data of all patients studied.

243. CARCINOEMBRYONIC ANTIGEN (CEA) IN TISSUE SECTIONS

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There is now evidence that levels of CEA may be elevated in colorectal cancer, as well as in pathologic states. Generally the procedure to measure the plasma levels of CEA is a commercially available radioimmunoassay. A relatively unexplored area is the detection and localization of CEA in conventionally prepared tissue sections.

The purpose of this study is: 1) To explore a laboratory method that can be applied in diagnostic pathology in the average community hospital. 2) To evaluate the levels of CEA in patients with colorectal cancer: preoperative measurement in patients who have undergone potentially curable surgery; serial measurements to monitor response to chemotherapy and radiotherapy in patients with unmeasurable disease. 3) Measurement of CEA in several other pathologic specimens to assist in the identification of the primary neoplasm in cases of metastatic cancer in which the primary tumor is not obvious.

244. CEA AND FHAP AS BREAST CANCER MANAGEMENT PARAMETERS

Torney, D. C., Larson, F. C., Crowley, J. J., Friedman, R. B., Entine, S. M., University of Wisconsin, Madison Campus, Medical School, Dept. of Human Oncology/Medicine, 450 N. Randall Ave., Madison, Wisconsin, 53706, United States of America
The summary for this project is under entry no. 23.

245. TUMOR MARKERS IN BREAST CANCER

Malkin, A., Kellen, J. A., Sunnybrook Hospital Medical Centre, Dept. of Clinical Biochemistry, 2075 Bayview Ave., Toronto, Ontario, M4N 3M5, Canada

A great deal of interest has been generated recently in the use of tumor markers in cancer detection and treatment. While their availability has become widespread, there is still considerable controversy regarding their precise usefulness. This constitutes an economic as well as a serious potential health hazard, leading, in the case of false positive tests, to unnecessary diagnostic and therapeutic intervention and in the case of false negatives, to a false sense of security.

Our studies on breast cancer lead us to believe that the carcinoembryonic antigen (CEA) test has predictive value for recurrence following surgery independent of conventional staging procedures and should perhaps be taken into account in stratifying patients into treatment arms for clinical trials and certainly in interpreting the results of these studies. To test this hypothesis, it was proposed that CEA testing be included in the diagnostic work-up and clinical follow-up of patients admitted to a comprehensive clinical study of breast cancer currently underway in the Metropolitan Toronto area.

In Trial I, which is essentially a comparison of CMF versus ovarian radiation in premenopausal women with primary breast cancer, 70 patients have been entered into the study. Trial II consists of a comparison of tamoxifen versus no tamoxifen in post-menopausal women with primary breast cancer; 141 subjects have been entered in this investigation. In addition, we are monitoring treatment in a group of premenopausal patients with metastatic disease receiving tamoxifen therapy to test its usefulness as a guide to subsequent treatment; 51 patients are included in this study. The data on the trial patients have not yet been analyzed with respect to recurrence or survival so that we are unable to draw any definitive conclusions at this time concerning the prognostic implications of the CEA test in these groups of patients nor the lead time to recurrence. However, in the Trial II study, there appears to be an unequal distribution between positive and negative CEA results among the patients stratified into local radiotherapy versus no local radiotherapy prior to randomization into tamoxifen versus no tamoxifen. If the bias is maintained, it will be interesting to see whether it influences the interpretation of the results of treatment.

246. IMMUNODIAGNOSIS OF GYNECOLOGICAL MALIGNANCIES

Kaupila, A., Oulu University Central Hospital, Dept. of Obstetrics & Gynecology, Kajaanintie 51, 90220 Oulu 22, Finland

OBJECTIVE: To evaluate the clinical value of carcinoembryonic antigen (CEA) and carbocyanide dye-binding polyan-

ion in the diagnosis of malignancies as well as in the follow-up of patients after treatments.

APPROACH: This investigation was started about two years ago and about 250 patients have been studied before, during and after treatment. CEA determinations have been made radioimmunologically (Reagents are supplied by F.Hoffman-LaRoche Diagnostica) and CPA photometrically.

PROGRESS: Preliminary results have been reported in the XIX Congress of Scand. Assoc. for Obstetricians and Gynecologists, Reykjavik, 1-4th June 1976. (Abstracts, p.49).

247. STUDY OF COLORECTAL CARCINOMAS WITH THE AID OF ANTI-CEA ANTIBODY-I-131 FOR A BETTER PROGRAMMING OF SURGERY AND FOLLOW-UP

Piat, G., Cangemi, V., Sopranzi, N., Di Tondo, U., Cavallotti, C., Fiori, E., Università degli Studi di Roma, Istituto Patologia Chirurgica, c/o Policlinico Umberto I, Rome, Italy

OBJECTIVE: A more precise surgical treatment and a selective lymphadenectomy.

APPROACH: Serial determinations of CEA levels and mapping of (pathological) lymph nodes by intravenous administration of anti-CEA antibody-I-131 and/or by administration of fluorochrome conjugated proteins.

PROGRESS: CEA concentrations in the metastasized lymph nodes in gastrointestinal carcinoma, directly related to the neoplastic involvement, have been determined, using in vitro fluorescent anti-CEA antibodies. In non-neoplastic gastrointestinal diseases regional nodes are CEA negative. In patients with gastrointestinal cancer, with elevated plasma concentrations of CEA, the non-metastasized lymph nodes were CEA negative. On the basis of these preliminary results, we are studying the possibility of utilizing this technique in vivo intraoperatively as an indication for enlarged lymphadenectomy.

248. IMMUNODIAGNOSTIC PARAMETERS FOR DETECTION AND FOLLOW-UP OF PATIENTS WITH COLORECTAL CARCINOMA

Matzku, S., Zoller, M., Deutsches Krebsforschungszentrum, Inst. für Nuklearmedizin, Im Neuenheimer Feld 280, D6900 Heidelberg, Federal Republic of Germany

Known immunodiagnostic procedures based on the cellular immune response to putative tumor-associated markers shall be improved according to the criteria of clinical diagnosis. Two procedures are considered to be promising. One of them is leucocyte migration inhibition assay in the panel modification. This modification consists of confronting patients' leucocytes with a panel of homologous tumor extracts. We have demonstrated that tumor bearing patients respond to more than 50% of extracts in most cases. Patients with local recurrences respond accordingly, while patients with metastases show significant decline in responsiveness. Radioimmunological determination of CEA, on the other hand, is capable of monitoring disseminated disease very reliably. A combination of both methods should lead to a comprehensive detection of post-surgical recurrences.

In addition, we plan to evaluate the use of the leucocyte migration test for discrimination between benign and malignant committed polyps of the colon.

249. A STUDY OF THE NATURAL HISTORY OF COLORECTAL CANCER - CLINICAL STUDY OF TUMOR MARKERS IN COLORECTAL CANCER

Blumgart, L. H., Wood, C. B., Gillis, C. R., Bond, M., University of Glasgow, Royal Infirmary, Dept. of Surgery, 84 Castle St., Glasgow G4 0SF, Scotland, United Kingdom

OBJECTIVE: To determine the clinical and practical value of two tumor markers, carcinoembryonic antigen (CEA) and pregnancy-associated alpha2 glycoprotein (alpha2PAG) in patients with colorectal cancer.

APPROACH: Serum CEA and alpha2PAG levels were measured preoperatively and at regular intervals postoperatively in patients with large bowel cancer. CEA was assayed using a radioimmunoassay double antibody technique. Alpha2PAG was assayed by an immunoassay method using the Laurell rocket technique. In the postoperative period, patients with two consecutively elevated CEA levels were submitted to a planned program of investigation for the detection of tumor recurrence.

PROGRESS: Of 404 patients who have been studied to date 199 underwent apparently curative surgery. In a follow-up period of 18-52 months, 40 patients developed two elevated

CEA levels and tumor recurrence was confirmed in 37. Two patterns of CEA elevation have been noted in patients with tumor recurrence: a fast rise with grossly elevated CEA levels recorded within 6 months of the first recording of an elevated level, and a much slower pattern of rise with only moderate elevations in serum CEA levels within 12 months of the first recorded elevation. This is of prognostic significance in that those patients with a fast rise in CEA levels had a much poorer prognosis. Elevations in alpha2PAG levels corresponded to the clinical course of patients with colon cancer but compared less favorably with CEA as a tumor marker.

250. CLINICAL EVALUATION OF THE HORMONAL RECEPTORS AND THE CEA TISSUE VALUES IN RECTAL CANCER

Mazzeo, F., Bucci, L., Del Rio, G., Università degli Studi di Napoli, Fac. di Medicina e Chirurgia, Dept. of Semeiotica Chirurgica, via Orazio 31, 80100 Naples, Italy

The summary for this project is under entry no. 157.

251. LOCALIZATION OF TUMORS BY RADIOACTIVELY LABELED ANTIBODIES TO CARCINOEMBRYONIC ANTIGEN (CEA)

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OBJECTIVES: (1) To evaluate and establish a technique for localization of primary tumors and metastases using radioactively labelled anti-CEA antibody, scintigraphy and emission tomography. (2) To study antibody preparations from different animal species, evaluating how different affinities affect - improve - this localization. (3) To determine if the new technique is more sensitive than x-rays and ordinary scintigraphy when used for detection of very small metastases.

APPROACH: Antisera to highly purified CEA from monkeys, sheep and rabbits are available. They will be absorbed with tissue containing NCA and BGP I to get a low antibody content to these substances which have antigenic determinants in common with CEA. After labelling with highly active, short-lived isotopes (123I, 131I), the anti-CEA IgG is injected into patients with advanced CEA-containing tumors. Localization of radioactivity is performed mainly with gamma emission tomography. Analysis of serum CEA and tumor CEA bound to the labelled antibody will be made as well as total tumor CEA measured by RIA and quantitative immunofluorescence.

PROGRESS: A large batch of high avidity and titer sheep anti-CEA IgG has been prepared. No cross-reactivity to normal colon antigen or bile glycoprotein is noted. Four patients have been injected with 123I-anti-CEA IgG without adverse effects. Emission tomography has been performed, which is a more sensitive technique than scintigraphy. Out of 12 known tumor sites, 5 were localized by the antibody. Nonspecific uptake is high in liver and sometimes in intestines.

252. CONCURRENT MEASUREMENT OF UDP-GALACTOSYLTRANSFERASE AND CARCINOEMBRYONIC ANTIGEN LEVELS IN CANCER PATIENTS

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The summary for this project is under entry no. 9.

253. CARCINOEMBRYONIC ANTIGEN IN DIAGNOSIS OF BOWEL CARCINOMA

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Contractor will evaluate role of the simplified CEA radioimmunoassay test in diagnosis of gastrointestinal, pulmonary carcinoma, and other malignant neoplasms. He shall finish analyzing data in Phase I, finish project proposed in Phase II, characterize CEA-like material secreted into human gastrointestinal secretion, determine CEA in sputum, collect colonic malignant tissue for different investigators in CEA, evaluate new diagnostic procedures for diagnosing large bowel cancer, determine value of simultaneous measurement of several fetal antigens, continue evaluation of CEA in patients undergoing chemotherapy, radiotherapy, and advanced gastrointestinal cancer and in patients following resection of gastrointestinal cancer with curative intent.

254. STUDY OF SERUM CEA AND CALCITONIN IN PATIENTS WITH MEDULLARY CARCINOMA OF THE THYROID

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The summary for this project is under entry no. 146.

255. PLASMA DETERMINATION OF ASSOCIATED ANTIGENS TO PRIMARY ADENOCARCINOMAS

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The 125CEA-anti-CEA system, when employed on plasma in increasing concentrations, turns out to be able to detect more than 50% of clinically primary adenocarcinomas.

This RIA system is used to monitor the extraction of CEA cross-reacting antigens from breast primary carcinomas (breast cancer antigen(s), BCA). The antisera raised against these antigens, and the 125CEA-anti-BCA cross-system, show the immunological differences between BCA and CEA.

The RIA 125CEA-anti-BCA system, when clinically employed, substantiates the hypothesis of the presence of antigens, more or less cross-reacting with CEA in primary adenocarcinomas. Therefore, CEA should no longer be considered as a general adenocarcinoma marker, but as a glycoprotein, extracted from adenocarcinoma liver metastases, cross-reacting with similar glycoproteins from primary adenocarcinomas and, to a greater extent, from their metastases.

Thus the CEA and BCA experimental models allow only a diagnosis of early cancer, but not organ localization. The latter is theoretically impossible with glycoprotein (antigen) cross-reacting with CEA, even though, as BCA, extracted from primary adenocarcinomas. In fact they always cross-react with similar glycoproteins (antigens), from primary adenocarcinomas and obviously, from their metastases.

BIBLIOGRAPHIC REFERENCES: A. Bartorelli, R. Accinni; *Current Trends in Tumor Immunology*: 233-252, 1979. S. Orefice, A. Bartorelli; *Current Trends in Tumor Immunology*: 253-268, 1979.

256. CARCINOEMBRYONIC ANTIGEN IN AN UNSELECTED POPULATION

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The adult population of Busselton, which is relatively closed and stable, has since 1966 been the subject of ongoing surveys. These have been marked by extremely high accession rates, in the order of 90%. The surveys entail health questionnaires, medical examinations and numerous biochemical and other health indices such as electrocardiograms. In particular, the occurrence of cancer and changes in smoking habits are under close scrutiny. The immunochemical method used for detection of carcinoembryonic antigen (CEA) in serum is a microradioimmunoassay appropriate for this study in that it requires only 0.25 ml of whole serum. CEA estimations on the over 60 year old segment of the 1969 sample revealed that, of 955 tested, there were 43 persons positive for CEA (cut-off level for positivity in this assay was 3 ng/ml); of these 43, 6(14%) were found to have a cancer of the type associated with CEA in blood. Of the 912 negative subjects, 18 (2%) had such cancers. Also cigarette smoking was found to be strongly associated with CEA in blood, and could have accounted for raised CEA levels in about one half of those with CEA but no demonstrable cancer. Testing of the 40-60 year old segment from the 1969 bleed brought the total tested to 2,372: there were 73 positive tests, about 3% of the population, and cancer occurred in 9 (12%) of these 73 subjects. The population was bled again in 1975 and 3,120 persons over the age of 21 have been resurveyed for the prevalence of CEA. Under new assay conditions, the 'cut-off' level for a raised CEA is 10 ng/ml; over 15% are greater than 10 ng/ml and 2.3% are greater than 20 ng/ml. The latter are currently being investigated for possible cancers. There is again a very strong association of smoking with high CEA levels; 37% of persons with serum CEA levels greater than 20 ng/ml are heavy smokers, this being almost three times

the prevalence of heavy smokers (13.5%) in the entire population.

257. CARCINOEMBRYONIC ANTIGEN (CEA) AND OTHER BIOLOGICAL MARKERS IN CANCER-PRONE FAMILIES

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Carcinoembryonic antigen (CEA) was studied in 224 direct-line members and 96 unrelated spouses from pedigrees manifesting the cancer family syndrome (heritable predisposition to adenocarcinomas of the colon and endometrium). Distributions of CEA per se showed Poisson-like properties and values were therefore transformed by taking square roots. Effects of age and duration of smoking were adjusted for by linear regression. Cancer patients and relatives at high genetic risk had a significantly greater mean CEA than relatives at low genetic risk. Surprisingly, unrelated spouses had mean levels of CEA which were strikingly similar to those in the corresponding cancer risk class of their direct-line mates. A highly significant intraclass correlation in CEA was also observed between the direct-line relatives and their spouses, whereas the intraclass correlation between direct-line full siblings was lower, and that between spouses of full siblings was negligible. These results suggest the existence of both genetic and connubial effects on CEA in the cancer family syndrome, presumably due to a common environmental agent acting in concert with genetic predisposition to oncogenesis. A battery of biological markers were studied among high risk members of certain pedigrees, and an index of cancer risk incorporating CEA, percentage of T lymphocytes, lymphocytic transformation, aryl hydrocarbon hydroxylase, and IgA shows good potential for delineating individuals susceptible to cancer. Agreement of the observed number of aberrant index scores with the expected number of deleterious-gene carriers based upon Mendelian segregation and the age distribution of the sample provides preliminary support for the identification of cancer risk utilizing multiple biological markers. Work continues.

258. CARCINOEMBRYONIC ANTIGEN IN FECES OF NORMAL SUBJECTS AND PATIENTS WITH CANCER OF THE COLON

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OBJECTIVE: To measure the levels of carcinoembryonic antigen (CEA) in feces of normal subjects and patients with cancer of the colon and determine whether these levels show an increase earlier and more consistently than plasma levels in patients. This would provide a convenient, reliable measurement of CEA levels for diagnosis of cancer of the colon.

APPROACH: CEA levels will be determined by means of RIA in feces and blood in a statistically significant number of smoker controls. The presence in serum of other oncofetal proteins known to be cancer markers will also be measured. CEA levels in serum and feces will then be determined in untreated patients, and the presence of tumor markers in serum will also be determined.

A standardized technique is being developed for the simple and reproducible determination of CEA levels in feces. In cancer patients the tumor size, degree of invasion, metastases, histologic type and length of development will be evaluated.

Once established, the fecal CEA values and presence of oncofetal protein in serum of normal subjects will be evaluated according to age, sex, and smoking habits and statistical average, standard deviation, t and p will be determined.

Results in cancer patients will be compared with normal values, with special attention to CEA levels. Values obtained in feces and serum will be compared to determine if there are significant increases in fecal CEA in patients and if so, whether this occurs earlier or more measurably in feces than in serum.

259. PATHOLOGY OF DIGESTIVE TRACT AND OTHER MUCOUS MEMBRANES

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We are continuing to study the immunopathogenesis of human digestive tract and other cancers by focusing on biological markers produced by benign and malignant mucous membranes. We are improving the clinical use of biological markers, CEA and others, in the management of patients.

Our studies emphasize the key role of the liver in regulating CEA and other circulating marker glycoproteins. We will study the mechanism by which CEA and other glycoproteins are cleared from the blood, bound to the hepatic cell and Kupffer cell and excreted, as well as its clinical implications.

We will systematically study glycoprotein markers (CEA, ZGM and alpha-1-acid glycoprotein) in benign, malignant and transitional mucosa by combining immunoperoxidase staining, 'mucus' stains including sialo- and sulfomucins, correlating these with tissue differentiation, thus providing the basis for an improved glycoprotein characterization of tumors. We will assess the usefulness of these tumor markers in pathological diagnosis, including distinguishing between undifferentiated and well differentiated tumors and undifferentiated adenocarcinoma and lymphoma. We propose a new method for quantitating tumor glycoproteins in histological sections of human tumor tissues using quantitative immunoelectrophoretic analysis.

Using a combined immunochemical and immunohistological approach and xenogeneic and monoclonal antibodies, we will search for new markers of digestive tract epithelial mucosa looking for markers which reflect differing cell types and varying differentiation.

260. STUDIES ON THE CARCINOEMBRYONIC ANTIGEN (CEA) OF THE HUMAN DIGESTIVE SYSTEM

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OBJECTIVE: To characterize the tumor site of the carcinoembryonic antigen (CEA).

APPROACH: Ongoing studies will employ the controlled enzymatic degradation of CEA so that small fragments can be examined in detail. This will involve the use of various enzymes, under different conditions of temperature, time and pH.

PROGRESS: To date, such fragmentation has been produced by limited proteolysis and has resulted in multiple fragments producing discrete bands on SDS-polyacrylamide gel electrophoresis in the size range from 10,000-30,000 daltons. Identical products have now been obtained from several CEA preparations tested to date, regardless of whether or not PCA has been employed in the isolation procedure. Each of the two major low molecular weight fractions, excised from the gels, have been able to produce virtually complete inhibition of binding between thoroughly absorbed anti-CEA antiserum and 125I-CEA. Purification of the major fragments on a larger scale, and further biochemical and immunological characterization of these fragments are currently in progress. In addition, minor fragments produced by the various procedures are under examination, and antisera to these various materials are being prepared in order to determine which will result in the production of a more highly specific antitumor antiserum for use in radioimmunoassay. Hence, the ultimate results of these studies may provide information concerning physicochemical and immunochemical properties of major tumor marker and result in more highly specific radioimmunoassays for clinical utilization. Moreover, the assays that may evolve may well have the capacity to differentiate between different types of circulating tumor molecules and markers of other pathologic states.

261. CEA AND OTHER TUMOR-ASSOCIATED SUBSTANCES IN BLOOD

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This is part of a broader project. A summary of this subproject is not available.

E. DIAGNOSTIC AND PROGNOSTIC APPLICATIONS OF AFP

262. DEVELOPMENT OF A RADIOIMMUNOASSAY FOR AN ONCOFETAL ANTIGEN OF POTENTIAL USE IN THE DIAGNOSIS OF HUMAN BRAIN TUMORS

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OBJECTIVE: We aim to develop a radioimmunoassay for a new oncofetal antigen identified in both fetal brain and human brain tumors.

APPROACH: Neonatally tolerized rabbits challenged with preparations of human fetal brain tissue have responded by producing antisera, which after absorption with adult brain tissue, recognize an antigen present only in fetal brain and human brain tumors. The antiserum will be used to prepare affinity columns with the purpose of isolating the antigen. This in turn will allow the development of a radioimmunoassay. The RIA will be used to put the potential of the antigen in early diagnosis of human brain tumors.

263. ESTABLISHMENT OF A METHODOLOGY FOR RADIOIMMUNOASSAY IN CANCER - BASIC PARAMETERS AND NORMAL VALUES OF CARCINOEMBRYONIC ANTIGEN AND AFP

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The summary for this project is under entry no. 238.

264. RADIOIMMUNOASSAY OF CEA AND AFP AS A SCREENING TEST IN THE SURVEY OF HEALTH OF WORKERS IN THE CHEMICAL INDUSTRY

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The summary for this project is under entry no. 237.

265. STANDARDIZATION OF ASSAYS FOR ALPHA-FETOPROTEIN FOR DETECTION OF NEURAL-TUBE DEFECTS AND FOR MONITORING GERM-CELL CANCERS

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PROJECT DESCRIPTION: Alpha-fetoprotein (AFP) radioimmunoassay (RIA) of maternal sera indicates that fetuses with open spina bifida can be screened for antenatally. The definitive test is an elevated level of AFP in midpregnancy amniotic fluid. About half of the seriously afflicted will die soon after birth, but many others will suffer a shortened life of institutional misery; crippled, incontinent for urine and feces, with excessive cranial pressure leading to progressive hydrocephalus, subnormal intelligence, and blindness. Two hundred and fifty thousand dollars could be spent per child to sustain minimal level of self-sufficient human dignity. The purpose of this project is for CDC to rapidly standardize these antenatal screening and diagnostic tests and prove them in a demonstrative epidemiological setting. Further, sensitive AFP assays can monitor for relapse of a therapeutic remission from germ-cell tumors.

266. DETECTION OF EARLY PANCREATIC CANCER - ROLE OF RIBONUCLEASE, PANCREATIC ONCOFETAL ANTIGEN AND OTHER TUMOR-ASSOCIATED ANTIGENS (ABBREV)

Tytgat, G. N., Teunen, A., Huijbregtse, K., de Vet, B., Hoek, F., Holtsma, H. F., Universiteit van Amsterdam, Dept. of Medicine, Spui 21, Amsterdam, Netherlands

The summary for this project is under entry no. 16.

267. THE VALUE OF SERUM AFP IN ASSAY IN DETECTING LIVER REGENERATION AND NEOPLASTIC TRANSFORMATION IN CHRONIC LIVER DISEASE (ABBREV)

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Moderately high values of serum alpha-fetoprotein (AFP) are encountered in a significant number of chronic liver disease cases.

At a basic level, the serum AFP value could be representative of the intensity of the regeneration of liver parenchyma. Alternatively, it may reflect merely the rate of dedifferentiation (not directly connected with the overall regeneration). However, subjects developing neoplasia may display a different pattern of evolution of the serum AFP concentration. If this proves correct, the AFP assay would have a prognostic value either for the evolution of liver regeneration or for the neoplastic transformation and would provide means to make the difference.

The program of work shall be to perform follow-up studies on the group of patients previously observed and to make repeated studies in all borderline cases. Such clinical studies will be correlated with liver radioisotope scintigraphy and liver needle biopsy for morphological information and with biochemical investigation of s-GOT, s-GPT, s-gamma-glutamyl-transpeptidase and the assay of HBsAg. All final reports will be elaborated with application of specially prepared computer programs.

268. PREALBUMIN SUBFRACTIONS IN BRAIN TUMORS

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OBJECTIVE: By the advancement of protein chemistry, oncofetal antigens have become the major problem in immunodiagnosis for cancer patients. In the central nervous system, acidic or basic 'brain specific proteins' are currently studied regarding their biological functions and their clinical applications. Our attempt is to determine and purify the oncofetal 'brain specific protein' or brain tumor specific protein in the mixed acidic proteins, prealbumin (PA) fraction, in order to make them clinically useful.

APPROACH: Step 1: Biochemical qualification and quantification of PA fraction and its subfractions are analyzed by 7.5 percent DISC-PAG electrophoresis and densitometric calculation; comparison was made between fetal-adult brain, brain tumors, other organs, and body fluid from sera, CSF, and cystic brain tumor. Step 2: Each PA subfraction is immunochemically identified, whether it is a known antigen or not. Step 3: Some of the PA subfractions with the oncofetal 'brain specific proteins' or brain tumor specific protein are purified and the antibody prepared for further immunochemical studies. Step 4: The specific antigens thus obtained are investigated for the clinical immunodiagnostic use.

PROGRESS: PA subfractions were analyzed biochemically in 226 cases, in which tissue PA was mainly composed of 5 fractions, PA-I through PA-V from the anode to the cathode, and each fraction was further subfractionated into the 2-3 bands, while fluid PA showed only one peak corresponding to the tissue PA-II'. Both brain tumor and fetal brain contained higher PA-III and lower PA-IV in concentration than did adult brain. Antibody to the whole PA fraction obtained from neuroblastoma contained neuroblastoma specific protein. We have been currently undertaking the isolation of the PA-III and the production of its antibody in gliomas, meningiomas, and neuroblastomas.

269. THE IMMUNOBIOLOGY OF ALPHA-FETOPROTEIN IN ONCOGENESIS

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It has been well documented that embryonic or fetal antigens can be identified in the sera of mice and humans with a variety of neoplasia. Although the study of fetal antigens has largely emphasized their identification for the clinical diagnosis of malignancy, recent information suggests that such fetal antigens may have direct influence on both general host immunocompetence and specific tumor immunity. In particular, it has

been demonstrated, using alpha-fetoprotein (AFP) purified from mouse amniotic fluid, that under specific experimental situations AFP may function as a suppressive agent for immune responsiveness. Unfortunately, the specificity of this suppression, its general role as an immune regulator, its association with other materials, and its possible function as a tumor promoting agent, are sources of controversy. In our laboratory, over the past year using select models of tumor induction, we have documented a broad, probably antigen non-specific, but perhaps organ specific, interaction of AFP at several levels. For example, treatment of mice with AFP appears to stimulate the production of a suppressor T cell population which markedly alters the natural history of Moloney sarcoma virus induced tumors. Furthermore, AFP administration influences both spontaneous leukemia in AKR/J mice and autoimmunity in New Zealand black mice. Similarly, in genetically susceptible mice (B6C3), AFP appears to be a co-promoter of diethylnitrosamine and dieldrin induced hepatomas. In contrast, AFP appears not to influence the induction of methylcholanthrene or DMBA tumors. Because these interactions of AFP appear to be multi-factorial, it is planned to further identify and define these select features, extend them to other models of oncogenesis, and in particular, to address organ and lymphocyte subpopulation specificity. These will be accomplished by: 1) Defining the influence of AFP on ontogenic influences of the immune response; 2) characterization, including Ly phenotyping, of the suppressor cell population generated by AFP; 3) identification of the mechanism of AFP acceleration of AKR/J leukemia; 4) study of the influence of AFP on reversibility of hepatic nodules; and 5) defining the additional mechanisms of AFP modulation of host responsiveness.

270. HUMAN TUMOR ANTIGENS - STUDIES IN HUMAN ALPHA1-FETOPROTEIN

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The major objective of the work and progress is to assess the immunoregulatory properties of human alpha1-fetoprotein (AFP) both in allogeneic and xenogeneic systems.

Human AFP will be purified by a number of different techniques. These will include the physicochemical approach to the purification of human AFP previously described in this laboratory, as well as a number of other techniques involving immunoprecipitation and affinity chromatography. It may well be that different AFP populations will be obtained by these different procedures so that some information may be forthcoming as to what specific subpopulation of AFP molecules, if any, is involved in immunoregulation. The systems of immunoregulation to be studied will include those involving MLR as well as lectin-stimulated lymphocytes. An attempt will be made to assess the involvement of the analogue of I-region triggering in the mouse vis-a-vis AFP immunoregulation. A number of studies will be undertaken, aimed at determining a structure-function relationship in the human AFP molecule.

In the studies completed, an RIA for AFP and its clinical utility have been described and a physicochemical technique for the purification of human AFP has been established. Employing ascites fluid as the starting source of material, 'monoclonal' human AFP has been obtained. Initial studies of this material indicate that human AFP preparations do not have universal immunoregulatory effects so that any such property would have to be attributable to either a subpopulation of molecules or to a contaminant of AFP preparations employed in other investigations. Further studies along these lines are in progress.

A variety of studies involving the assay of AFP in the circulation of pregnant women have led us to caution against the indiscriminate use of circulating maternal AFP as an indication for abortion during the later part of pregnancy or as a method for 'ruling out' neural tube defects (NTD) at any time during pregnancy. The possibility of such an assay as an adjunct in the diagnosis of NTD still remains a possibility.

F. DIAGNOSTIC AND PROGNOSTIC APPLICATIONS OF OTHER TUMOR-ASSOCIATED ANTIGENS

271. HETEROANTISERA TO HUMAN LEUKEMIA-ASSOCIATED ANTIGENS

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Our project has provided evidence to date that leukemia-associated antigens are detected on human leukemic myeloblasts by hetero and alloantisera. The distinction between these antigens and histocompatibility and other non-leukemic stem cell antigens has been extensively studied.

We have isolated and partially purified a characteristic glycoprotein antigen shed from human myeloblasts in short-term *in vitro* culture.

We have used heteroantisera to these antigens to reliably detect imminent leukemic relapse by immunofluorescence assays of bone marrow specimens from patients with acute myeloblastic leukemia in remission.

Our plans are as follows: 1) To continue to develop and refine heteroantisera to human leukemia-associated antigens with specificity for morphologically characterized leukemic myeloblasts. Specific primate antisera and monoclonal murine hybridoma antisera will be prepared to highly purified glycoprotein antigen and to its subunits. 2) To continue characterization of the biochemical nature of human leukemic myeloblast-associated antigens. Antigenic compounds obtained by gel chromatography and isoelectric focusing will be purified to apparent homogeneity by two-dimensional electrophoresis with determination of amino acid and carbohydrate composition. 3) To continue to develop and refine clinically useful criteria for detection, characterization and enumeration of leukemic myeloblasts. Circulating soluble leukemic antigen in serial samples of patient's serum will be quantitatively assessed by radioimmunoassay and ELISA techniques.

272. IMMUNODIAGNOSIS OF LEUKEMIAS AND LYMPHOMAS

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OBJECTIVES: Evaluate cellular antigens of human lymphoid organs that characterize lymphoid cells at early stages of differentiation, especially in the bone marrow and thymus. Perform functional analysis of cells early in differentiation in bone marrow and thymus, using antisera directed against thymus antigen 1 (TA1), as well as TA2, TA3, null antigen, and surface and cytoplasmic immunoglobulin. Analyze possible helper and suppressor cell activities by cells bearing the above antigens. Correlate newer markers with those of more mature lymphocytes. Perform studies to identify additional subgroups on the basis of cellular phenotype, using new thymus-derived and null antigens and an available patient population of approximately 150 per year. Surface marker data will be correlated with clinical features, response to therapy, and survival.

273. IMMUNODIAGNOSIS OF LYMPHOMAS

Udayachander, M., Meenakshi, A., Michael, A., Muthiah, R., Cancer Inst., Dept. of Biochemistry, Adyar, Madras 600020, Madras, India

OBJECTIVE: To facilitate the diagnosis of lymphomas in asymptomatic stage. The presence of a circulating lymphoma-associated antigen (LAA) has been demonstrated only in lymphoma patients by various immunological methods such as agar gel diffusion, immunoelectrophoresis and counter-electrophoresis. So far the sera of 152 normals, 496 cases of various types of cancers and 213 cases of confirmed lymphomas have been tested by these methods. These studies have revealed that a specific LAA anti-LAA system is possibly operative in lymphoma patients. At present this immunodiagnostic test is routinely employed to screen patients with such symptoms as low-grade fever, general debility and generalized lymphadenopathy. This study has shown promising results in that in 8 cases, the LAA test was reactive at the time of presentation when the histopathological diagnosis was inconclusive due either to the lack of representative specimens or to the non-availability of a sizable

lymph node. In such cases, the histopathology later became confirmatory.

274. CHARACTER OF LYMPHOCYTE DIFFERENTIATION ANTIGENS

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Human B cells express a multiplicity of cell surface antigens that are useful markers for identifying different subpopulations of bone marrow-derived (B) cells and their malignant counterpart in leukemias and lymphomas. We have prepared an anti-human B-cell antiserum (BDA) that reacts with one or more differentiation antigens on B-cells from all normal lymphoid compartments. The antigen(s) is distinct from other known cell surface constituents on human B cells (i.e., Ig, Fc, C, Dr). Of clinical interest is the observation that this antiserum reacted with the majority of neoplastic lymphocytes from some leukemic patients (both acute and chronic type), even though these lymphocytes expressed little or no surface immunoglobulin (a usual characteristic of B lymphocytes) and did not form spontaneous E-rosettes (a marker for identifying T cells). This reagent thus has potential clinical application for identifying a determinant that serves as a marker for certain lympho-proliferative malignancies.

In this study, we propose to use immunochemical techniques to first isolate and further characterize the nature of the B cell antigen(s) on normal and leukemic B cells that react with this specific anti-B-cell antiserum (BDA) and second, isolate and identify other B-cell differentiation antigens. The methodology to identify and characterize lymphocyte differentiation antigens is developed and we have employed these methods to identify two differentiation antigens on normal and cultured human thymus-dependent (T) lymphocytes. We plan to use the same strategy for further characterizing the B-cell antigen(s) reacting with this specific B-cell antiserum as well as other conventional and monoclonal B-cell antisera.

275. CLASSIFICATION OF HUMAN LEUKEMIAS BY QUANTITATION OF TUMOR ANTIGENS

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This is part of a broader project. A summary of this subproject is not available.

276. ANTIGENS ASSOCIATED WITH BREAST

CARCINOMA - BCA (BREAST CANCER ANTIGEN(S))

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OBJECTIVES: Extraction, purification, isolation, physicochemical characterization and localization at the cell level of antigens associated with breast carcinoma, absent in normal or dysplastic control breast tissue.

APPROACH: The extracts of 48 breast carcinomas were studied by RIA techniques (125I CEA-antiCEA system): a different immunological CEA-like activity was detected in each of them. These carcinomas were classified into 5 groups according to their increasing specific activity (U/microgram): 1) not detectable specific activity; 2) specific activity between 0 and 0.5; 3) specific activity between 0.5 and 1; 4) specific activity between 1 and 3; 5) specific activity greater than 10. Only 6% of the 48 carcinomas examined belong to group 5. These findings, though remarkably reducing the possibility of employing all primary breast carcinomas for the preparation of one single BCA (Breast Cancer Antigen), on the other hand, open new and interesting possibilities of research. In fact, it could be possible to purify and isolate different antigens associated with breast carcinomas, and thus to employ not only one, but many BCAs as tumor markers for a diagnostic test.

PROGRESS: BCA 5 is extracted and partially purified from 10 g of primary breast carcinoma (from group 5) with a 6.4% yield, and a specific activity of 1,600 U/microgram. At this purification step, BCA is labelled with (125I)Na. The subsequent G-200 Sephadex gel filtration points out 3 labelling peaks, eluted from the column before the free iodine. By means of anti-BCA antibodies, absorbed and not absorbed (in collaboration with Dr. A. Bartorelli), the immunological activity is detected

only on the first labelling peak. By employing bioabsorption chromatography with anti-normal breast tissue antibodies and normal human antiserum conjugated to Sepharose 4B, the 2nd and 3rd labelling peaks are identified as normal serum and tissue contaminants and, consequently, discarded. This step, which turned out very useful for the purification, will be adopted, before labelling, in a future BCA preparation.

277. SIGNIFICANCE OF GP48 IN DIAGNOSIS OF BREAST CANCER

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The objective of this proposal is to characterize and to quantitate those glycoproteins which are synthesized and released by human breast cancer surgical specimens. Special emphasis is given to gp48 and related glycoproteins, which appear to be released in greater abundance from malignant than benign specimens.

To achieve this, we propose to biosynthetically label glycoproteins with 3H- or 14C- carbohydrate precursors using either established cell lines or organ cultures of human breast cancer surgical specimens. The labelled glycoproteins are characterized by two-dimensional gel electrophoresis. Those components which are related to known serum glycoproteins are immunoprecipitated and their antigenic relationships are further investigated. Glycoproteins which appear antigenically unique will be purified from surgical specimens, using the biosynthetically labelled glycoproteins as tracers. Their relationship to normal membrane components derived from breast epithelial cells will be investigated using human milk fat globular membranes. The purified glycoprotein will be used for the production of immune antisera or monoclonal antibodies. After appropriate adsorptions, the specific antisera will be used to establish a quantitative immunoassay using either a nonradioactive immunodiagnostic procedure with rate nephelometry or a radioimmunoassay with the 125I labelled glycoprotein.

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278. ANTIGENS OF HUMAN OVARIAN TUMORS

Lloyd, K. O., Old, L. J., Lewis, J. L., Welander, C. W., Sloan Kettering Inst. for Cancer Research, 410 E. 68th St., New York, New York, 10021, United States of America

Ovarian cancer is second only to breast cancer as the most common cause of death from gynecological malignancy in women in this country. The principal objective of this project is to identify antigens or markers of ovarian tumors which can be used in an immunodiagnostic assay for the detection of early ovarian cancer. Xenogenic sera will be used to identify (1) antigens in extracts of ovarian tumors and (2) cell surface and shed antigens of cultured ovarian tumor cell lines. Using rabbit antisera we have already identified two antigens (OvC-1 and OvC-2) present in ovarian tumors but not in normal ovaries. Both these antigens are present in amniotic fluid which is a convenient source of material for isolation of the antigens. OvC-1 is a new pregnancy-associated antigen. OvC-2 is a new onco-fetal antigen and is found in a fetal intestine as well as in ovarian tumors. The development of sensitive assays (e.g. radioimmunoassays) capable of detecting these antigens in serum or plasma will enable us to determine their possible usefulness for the immunodiagnosis of ovarian cancer. Cultured ovarian tumor cells will be used in attempts to identify new antigens not previously identified when tumor extracts were used. Components shed from cultured ovarian tumor cells will also be studied. Antisera to the cultured cells and their released components will be raised in rabbits and monoclonal antibodies will be produced using the procedure of Kohler and Milstein. The specificity of the antisera will be studied by serological techniques using binding or adherence assays and by radioimmunoprecipitation. New antigens showing ovarian tumor

specificity will be examined for their possible use in the early detection of ovarian cancer.

279. IMMUNODIAGNOSIS OF HUMAN OVARIAN CANCER

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OBJECTIVE: Ovarian cancer is an occult neoplasm which usually remains undetected until the curative stage has passed. We have been investigating ovarian tumor-associated antigen NB/70K with the intention of developing a radioimmunoassay capable of detecting tumor antigen in plasma before clinical signs of disease are apparent.

APPROACH: We have purified human ovarian tumor-associated antigen NB/70K from surgical specimens of ovarian epithelial tumors. NB/70K appears to be a tumor-associated antigen which has no cross-reactivity with carcinoembryonic antigen and which has an apparent molecular weight (as estimated by SDS acrylamide gel electrophoresis) of approximately 60-70,000 daltons. We propose to develop a radioimmunoassay sensitive enough to detect NB/70K in plasma. Once the assay has been developed, we will examine plasma samples from groups of patients with ovarian tumors, non-ovarian tumors, benign gynecological disease, benign nongynecological disease as well as a normal control population so that the selectivity and specificity of the assay can be determined. We also propose to investigate the use of the assay for staging and monitoring the progression of disease in patients being treated for ovarian cancer.

280. ANTIGEN MARKERS IN DIAGNOSIS OF PROSTATE CANCER

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The objective of this proposal is to continue our study on antigen markers which may be useful in the detection of prostate cancer. A new, specific and sensitive solid-phase immunofluorescence assay for acid phosphatase is to be evaluated for its potential value in the early diagnosis of prostate cancer. A prostate-specific antigen has been purified to homogeneity. Xenantisera have been raised against the prostate antigen and the specificity of the antisera will be determined. Initial data indicate that this antigen may be of potential use in identifying prostate cancer patients. A new sensitive immunoassay, such as radioimmunoassay or enzyme immunoassay, will be developed and evaluated. In addition, a prostate tumor-specific antigen has been demonstrated and antiserum against it has been produced in non-human primates (baboon). Isolation, purification and characterization of this prostate cancer antigen will be performed. Furthermore, a prostate acid phosphatase-binding globulin has been isolated from normal human serum by affinity chromatography using purified prostatic acid phosphatase. Its potential clinical application in prostate tumor radiolocalization and immunotherapy will be studied. Finally, an immunoassay for measurement of serum ribonuclease is being developed, as purification of two prostatic ribonucleases has been achieved.

281. ANTIGENIC COMPONENTS OF HUMAN PROSTATIC ADENOCARCINOMA

Rose, N. R., Pontes, J. E., Choe, B., Morrison, M. K., Wayne State University, School of Medicine, Dept. of Microbiology & Immunology, 1014 Rivard, Detroit, Michigan, 48207, United States of America

Immunological approaches to human prostate cancer depend upon the existence of cell-surface antigens capable of evoking a specific immunological response. The purpose of our study is to determine whether patients with prostatic cancers develop an immunological response to antigens unique to their tumor cell surfaces. In addition to providing information about the fundamental cellular changes underlying malignant transformation, these studies have the immediate practical goals of providing the means to assess the immunological response of patients to their tumors during the course of disease both with and without treatment; of opening avenues for manipulating the immunological response in favor of the tumor-bearing host; and of developing immunological methods for the earlier and more accurate detection of prostatic cancers.

Carcinoma of the prostate provides an excellent model for studying cancer immunology in man because of its relatively high prevalence and chronic nature. It is a tumor whose progress can be slowed down by surgery and, in some cases, by hormonal therapy or chemotherapy. Because patients with prostatic carcinoma are in relatively good health during much of the course of their disease, they have an opportunity for developing an immune response to the tumors.

We plan to continue our studies in prostatic carcinoma by evaluating the specific immunologic response of patients to their own tumors. Cell-mediated as well as humoral responses will be assessed by several new approaches. These include in vivo and in vitro cytotoxicity assays as well as biochemical studies of prostatic tumor specific antigen. These investigations should provide a rational basis for immunotherapy aimed at modifying the response to this cancer in man.

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282. IMMUNODIAGNOSTIC MEASURES IN CERVICAL CANCER

Kessler, I. I., Aurelian, L., University of Maryland, Baltimore Professional Schools, School of Medicine, Dept. of Epidemiology & Preventive Medicine, 655 W. Baltimore St., Baltimore, Maryland, 21201, United States of America

The objective of this study is to systematically assess the reliability and validity of the AG-4 Serological Test in predicting the subsequent course of cervical cancer. This tumor-specific antigen has been shown to be present in most of the patients with invasive cervical cancer or with recurrent cervical cancer, to be virtually absent in controls without cervical disease and to be present in a minority of patients with pre-neoplastic cervical disease. Patients in various stages of cervical neoplasia are being followed prospectively in order to measure their blood levels of AG-4 before, during and after invasion.

283. ULCERATIVE LESIONS AND TUMORS - PAPILLOMAS OF ORAL CAVITY

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Papillomavirus antigens were detected in verruca, multiple papillomas, condylomata and laryngeal papillomas of the oral cavity by the peroxidase-antiperoxidase technique.

BIBLIOGRAPHIC REFERENCES: Jenson, A.B., Rosenthal, J.R., Olson, C., Pass, F., Lancaster, W.D., and Shah, K.: Immunologic relatedness of papillomaviruses from different species. *JNCI* 64(3): 495-498, 1980.

284. HL-A AND CANCER OF THE OESOPHAGUS IN SOUTH AFRICAN NEGROES

Hammond, M. G., Brain, P., Angorn, I. B., Appadoo, B., Natal Inst. of Immunology, Transplantation Unit, P.O. Box 2356, Durban 4000, Natal, South Africa

This is a follow-up study of a previous investigation (Hammond et al., *Tissue Antigens* 9, 1-7 1977) in which a correlation was found between patients with cancer of the oesophagus and the HL-A antigen BW45. (The original study found that 16 of 101 patients with cancer of the oesophagus had the antigen BW45 compared to 36 of 500 controls.

285. IMMUNOLOGICAL MARKERS OF TUMOR TYPE SPECIFIC ANTIGENS IN GASTROINTESTINAL CANCER

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PROJECT OBJECTIVES: 1) To test the diagnostic potential of the tube modification of the Leukocyte Adherence Inhibition test (LAI) for gastrointestinal cancer. 2) To evaluate if the

mechanism involved in LAI can reflect upon tumor resistance in tumor patients or healthy individuals.

PLAN: Applying a modification of Thompson's LAI test to patients with colorectal carcinoma other neoplasms and to controls in which tumor extracts from primary and tissue culture colon cancer and urogenital cancers are used.

METHOD: Culture and preparation of homogenates from the HT-29 cell line and surgical specimen. Protein concentration determinations by the Lowry method. Separation of peripheral WBC's from heparinized blood samples. Test tube adherence and quantitation of percentage of adherence by the ZBI Coulter counter determination of non-adherent cells.

FINDINGS TO DATA: Percentage of nonadherence responses were significantly higher in cured colorectal cancer and polyp of the colon than in other patient groups inclusive of advanced colorectal cancer. The Nonadherence index did not demonstrate specificity for colon cancer but specificity for urogenital tract cancer is suggested.

286. HLA ANTIGENS IN PATIENTS WITH INTESTINAL POLYPS

Illeni, M. T., Clerici, E., Pellegris, G., Spinelli, P., Pizzetti, P., Pasquali, M., Istituto Nazionale per lo Studio e la Cura dei Tumori, I Via G Venezian, 20133 Milan, Italy

OBJECTIVE: Some genes involved in the pathogenesis of tumors are believed to be contained in the greater complex of histocompatibility, closely associated with the genes which control the expression of HLA antigens. An increased incidence of some HLA antigens in a given neoplastic form can thus indicate the existence of a 'linkage disequilibrium' of these genes with the genes involved in the pathogenesis of the neoplasm.

The phenomenon can be used clinically to select a high-risk population for a given tumor.

APPROACH: HLA typing, indicated in protocol 4/1 of 1976, is in progress. The lymphocytotoxicity test is used.

We will also look for correlations with blood group ABO, Rh, Lewis, the secretory nature of group-specific substances, as well as histological type. Familial studies will also be conducted.

PROGRESS: Fifty-two cases of non-familial single and multiple polyps have been typed up to now.

A highly significant increase was observed in the HLA-Bw22 antigen in the patients as compared to the controls (p equals 0.00006). (Translated)

287. A STUDY OF THE NATURAL HISTORY OF COLORECTAL CANCER - CLINICAL STUDY OF TUMOR MARKERS IN COLORECTAL CANCER

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The summary for this project is under entry no. 249.

288. A STUDY OF HUMAN PANCREATIC DUCT ANTIGEN

Gold, D. V., University of Kentucky, School of Medicine, Dept. of Pathology, Limestone & Euclid, Lexington, Kentucky, 40506, United States of America

A pancreatic duct antigen will be studied to determine the potential of the antigen as a marker of pancreatic tissue and pancreatic carcinoma in particular. The pancreatic duct antigen will be purified from specimens of normal and neoplastic pancreas and characterized as to amino acid and carbohydrate composition. Antisera will be raised in rabbits and the specific antibody purified by absorption and affinity chromatography. The tissue and tumor specificity of the antisera will be analyzed by immunoperoxidase procedures in a retrospective study of biopsy specimens. A radioimmunoassay will be constructed to compare immunologic relatedness between normal and neoplastic pancreatic duct antigen. Promising antisera will be analyzed for potential use in the immunodiagnosis of pancreatic cancer by quantification of circulating antigen.

289. TUMOR ANTIGENS IN PANCREATIC CANCER

Chu, T. M., Douglass, H. O., Loo, R. M., Inaji, H., Roswell Park Memorial Inst., 666 Elm St., Buffalo, New York, 14263, United States of America

The objective of this proposal is to isolate and purify tumor antigens from pancreatic cancer. The tumor antigens are to be isolated from plasma membrane fraction of the pancreatic

tumors, from the extracellular fluid (ascites fluid) and tumor tissues of pancreatic cancer patients. The antigens (free or in complexed form) will be purified by gel filtration, ion-exchange chromatography, affinity chromatography, immunoadsorbent technique, preparative polyacrylamide gel electrophoresis and isoelectric focusing. The chemical, physical and immunological characteristics of the antigens will be studied. Xenobiotic antisera will be raised with the purified antigens and specificity of the antiserum studied. If biochemical and immunologic data indicate that the antigens are specific for or associated with pancreatic cancer, immunodiagnostic procedures will be developed which can be of value in early detection of pancreatic cancer and in monitoring the efficacy of therapy currently available for pancreatic cancer patients. During the past two years of this grant period, a glycoprotein antigen, shown not to be a normal serum component, has been isolated from the ascites fluid of pancreatic cancer patients. A plasma membrane fraction has been prepared from pancreatic tumors which demonstrates a pancreas tumor-associated protease(s) activity. This plasma membrane fraction also contains antigen component(s) which has been used successfully in a leukocyte adherence inhibition assay specifically for diagnosis of pancreas cancer. Pancreatic tumors have been shown to contain perchloric acid soluble antigenic species different from those of colonic CEA. Further, IgM- and IgG-containing immune complexes have been identified in pancreatic cancer patients. The purpose of this renewal proposal is to continue our study of these pancreas cancer-specific or -associated tumor markers and their clinical application.

290. PURIFICATION OF TAA IN HUMAN BRONCHOGENIC CARCINOMA

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OBJECTIVE: To purify TAA associated with bronchogenic carcinoma, to develop a sensitive assay for it using specific rabbit antiserum, and to investigate the possible use of this assay in immunodiagnosis.

APPROACH AND PROGRESS: We have purified a common TAA from human bronchogenic carcinoma using a specific antiserum raised in rabbits to monitor purification. We have adapted the ELISA assay for this purpose. Preliminary studies show that the sera from patients with early lung cancer inhibit the ELISA test we have developed. The validity of this observation is being investigated.

291. ANTIGENS AND IMMUNITY IN HUMAN LUNG CANCER

Bell, C. E., Washington University, School of Medicine, Dept. of Pathology/Medicine, 660 S. Euclid Ave., St. Louis, Missouri, 63110, United States of America

The overall objective of the proposed research is to identify parameters which can be measured to provide earlier and more accurate diagnosis, and ensure improved treatment, management, and assessment of prognosis of patients with lung cancer. It is planned to accomplish this through the following specific methods and objectives: 1. To identify human lung cancer plasma membrane tumor-associated antigens (TAA's) which are absent from normal adult tissue and from cancers of other organs; to determine the occurrence of these TAA's in the different histologic types of human lung cancer and in various premalignant conditions of the respiratory tree. One such antigen associated with oat cell carcinoma of the lung has been identified. Other TAA's will be identified by two different approaches. The first involves planned immunization of rabbits and monkeys with human lung cancer plasma membranes and assay of the immune sera by indirect immunofluorescence assay. The second utilizes the host's own immune response to his tumor: appropriately treated host sera will be assayed by indirect immunofluorescence; host leukocyte reactivity to crude lung cancer antigens will be assayed by leukocyte adherence inhibition. 2. To determine the occurrence and concentration of these TAA's in the sera and respiratory secretions of patients with various malignant, premalignant, and benign conditions. The TAA's will be solubilized and purified, and a radioimmunoassay developed to determine the above. 3. To determine the occurrence of cellular and humoral immunity to these defined

TAA's, and of specific blocking factors related to these TAA's in patients with various malignant, premalignant, and benign conditions. A leukocyte adherence inhibition assay and a double antibody primary binding assay will be developed to determine the above. 4. To determine the relationships of the occurrence of these parameters to the different stages of human lung cancer.

292. ISOLATION OF SOLUBLE PURIFIED TUMOR-ASSOCIATED ANTIGENS OF HUMAN MALIGNANT MELANOMA

Suter, L., Macher, E., Westfälische Wilhelms Universität, Hautklinik, Experimentelle Dermatologie, Von-Esmarch-Strasse 56, D4400 Münster, Federal Republic of Germany

OBJECTIVE: Preparation of soluble purified tumor-associated antigens of human malignant melanoma that can be used for tests of cell-mediated and humoral immunity and for the determination of circulating tumor-associated antigens in melanoma patients.

APPROACH: Mainly tissue and membrane preparations from excision-biopsies of human malignant melanoma will be used for the extraction of tumor-associated antigens. Smaller preparations are planned from long-term cell lines of malignant melanoma. The extracts will be purified by salt precipitation, ion-exchange chromatography, and gel electrophoresis. Antigenic activity will be monitored with heteroantisera of rabbits and monkeys by the following assay methods: 1) immune precipitation; 2) passive hemagglutination; 3) radioimmunoassays; and 4) counter-current electrophoresis.

Purified preparations of antigens will be characterized by quantitative determinations of neutral sugar, amino sugar, and protein.

The occurrence of the isolated and characterized antigens in normal and inflammatory tissue, in embryos, and in different malignancies will be tested.

The clinical relevance of the isolated antigens will be determined by comparison of individual in vitro results of immunity tests with the clinical course of the patients. Leucocyte migration assay, adsorption of cytotoxic lymphocytes by immunoadsorbents prepared with isolated antigens, and determination of circulating antigen levels by radioimmunoassays are planned.

PROGRESS: Antigen was extracted with the non-ionic detergent NP-40 from membranes of 3H-leucine labelled cultured melanoma cells. Antigenic activity was assayed with sera from melanoma patients.

293. IMMUNODIAGNOSIS OF MELANOMA

Seigler, H. F., Stuhlmeier, G. M., Duke University, School of Medicine, Dept. of Surgery, Box 3711, Durham, North Carolina, 27710, United States of America

Our primary objective for this project is the development of a sensitive radioimmunoassay for the detection of soluble human melanoma tumor associated antigens (TAA). Purified TAA and antisera specific for these TAA are required. We have produced a battery of human anti-melanoma alloantisera and non-human primate antisera which, once suitably absorbed, are operationally monospecific for human melanoma TAA. Using these antisera to monitor for pronase-solubilized melanoma TAA, we have performed preliminary purifications of these TAA from other cell membrane constituents such as HLA and Dr antigens and B-2 microglobulin. We plan to continue purification of these TAA using antibody and lectin binding techniques. Once purified melanoma TAA are obtained, they will be employed as antigen standards in the RIA and used to immunize mice. Spleen cells from these immunized mice will then be used to produce monoclonal anti-melanoma TAA antibody-secreting hybridomas. In addition, we are attempting to produce hybridomas between murine myeloma cells and B-enriched lymph node cells from a chimpanzee which has been hyperimmunized against human melanoma. Useful monoclonal antibodies from these two sources will be used in the RIA and to assist in further large-scale TAA purifications. We will evaluate the usefulness of the RIA for detection of serum-borne melanoma TAA in melanoma patients. The RIA will be of potentially great benefit in three ways: 1) using the RIA we may be able to detect recurrent tumor growth in melanoma patients prior to its clinical manifestations, 2) the RIA could be used to monitor the

response of melanoma patients to various therapeutic modalities, and 3) the RIA could be used for basic studies into the role of soluble TAA, free or in the form of immune complexes, on the host-tumor interaction.

294. DEVELOPMENT OF IMMUNODIAGNOSTIC TESTS FOR CANCER

Ibrahim, A. N., Georgia State University, School of Arts & Sciences, Dept. of Biology, 33 Gilmer St. S.E., Atlanta, Georgia, 30303, United States of America

OBJECTIVES: Increase the specificity and the sensitivity of an immunodiagnostic test for cervical and head and neck cancer. Purify the tumor associated antigens. Prepare specific antisera against these antigens. Periodically monitor the test by testing aliquots of sera from the same serum panel provided by NCI. An appropriate clinical evaluation of the immunodiagnostic test shall be done when the test is refined to the satisfaction of the Immunodiagnosis Program.

295. CELL SURFACE ANTIGENS OF CHILDHOOD BRAIN TUMORS

Zelter, P. M., University of Texas, Health Science Center at San Antonio, Medical School, Dept. of Pediatrics, 7703 Floyd Curl Dr., San Antonio, Texas, 78284, United States of America

OBJECTIVE: The overall objective of this project is to develop specific and sensitive serologic assays for brain tumor antigens which may provide improvements over techniques currently available for diagnosis, classification and monitoring of these neoplasms in children.

APPROACH: CSA (tumor related, fetal, differentiation type) of these brain tumors will be identified and later characterized, using serologic and molecular techniques which were developed in this laboratory. Next, three types of investigations using these CSA will be implemented: 1) determination of tumor CSA phenotype, 2) quantitation of soluble CSA in cerebrospinal fluid, serum, and/or urine by radioimmunoassay, and 3) determination of human anti-CSA antibody responses. The value of these tests for diagnosis, prognosis, and therapeutic assessment will be determined by correlation with clinical parameters.

296. PRIMARY ADENOCARCINOMA ASSOCIATED ANTIGENS - RADIOIMMUNOASSAY IN PLASMA

Barisorelli, A., Biancardi, C., Mistretta, R. F., Università degli Studi di Milano, Facoltà Medicina e Chirurgia, Via F. Sforza, 35, 20122 Milan, Italy

OBJECTIVES: Development of RIA techniques for the detection of plasma antigens associated with primary breast carcinomas.

APPROACH: Extraction and purification of CEA-like antigens from primary breast carcinomas (in collaboration with Dr. R. Accinni). Antisera preparation; labelling of these antigens; RIA development.

PROGRESS: Anti-crude-breast-BCA antisera have been prepared, which when absorbed with normal human plasma and breast non-malignant glandular tumor tissue extracts, recognize (125I)BCA, prepared by labelling purified BCA (Dr. R. Accinni).

297. RELATION OF BLOOD GROUP AND HUMAN TUMOR ANTIGEN

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Membrane-bound blood group determinants have been characterized as being carried by sets of glycosphingolipids (e.g., Aa, Ab, Ac; H1, H2, H3, etc.) and by carbohydrate chains bound to the hydrophobic membrane proteins such as 'band 3' protein. Alterations in the structure of the carrier portion as well as the terminal determinants are associated with development and with cancer. Specifically, we have detected a progressive branching of the carrier carbohydrate chain during development of erythrocytes. In human cancer, two types of significant changes in blood group determinants have been noticed; namely, the appearance in human cancer of incompatible blood group antigens foreign to the host, and the incomplete synthesis of blood group determinants and the associated accumulation of precursor chains.

This application is to propose studies on: 1. Characterization of structure in detail and variation of the membrane-bound blood group ABH, Lewis, Ii and Forsman antigens and their carrier molecular species. Particular focus will be made on the determinants carried by gangliosides, polyglycosylceramides and hydrophobic membrane protein ('band 3' or alike). 2. Further studies on ontogenetic changes in blood group carrier carbohydrate chains and a possible change of Forsman antigens during ontogenesis of endodermal organs. 3. Chemical and immunological characterization of the 'incompatible' blood group or Forsman isoantigens in human cancer and their corresponding isoantibodies present in normal and patients' sera. Particular emphasis will be the characterization of A-like antigen in tumors of O or B, and further extensive studies on Forsman antigen in tumors of F(negative) individuals in various cancers. 4. Enzymatic basis for synthesis of 'incompatible' blood group and Forsman isoantigens and for the process of i to I or I to i conversion.

298. CEA AND OTHER TUMOR-ASSOCIATED SUBSTANCES IN BLOOD

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The summary for this project is under entry no. 261.

299. NUCLEOLAR ANTIGENS OF HUMAN CANCER CELLS

Busch, H., Baylor College of Medicine, School of Medicine, Dept. of Pharmacology, 1200 Moursund Ave., Houston, Texas, 77025, United States of America

The objectives of these studies are (1) to purify and characterize a nucleolar antigen which has been found in a broad range of human malignant tumors, (2) investigate the function of the antigen by initially localizing it in the nucleolus and assessing its effects on a variety of nucleolar enzymes and other components as well as its origin, and (3) to evaluate the potential immunodiagnostic use of the antigen by a number of techniques.

The specific aims of these studies are (1) the separation, isolation and purification of these antigens and preparation of the corresponding monospecific antibodies; (2) inasmuch as preliminary studies have indicated that the antigen(s) is localized to perinucleolar chromatin and intranucleolar chromatin in association with the fibrils and beads of the 'nucleolonemas', one goal of these studies is to determine what function the antigen has in these special locations; (3) to investigate the antigen in large series of specific tumors and determine relationships of growth rates and amounts of antigen present; and (4) to determine whether the antigen is related to growth, and whether it is a fetal gene product or a product of exogenous genes.

300. PURIFICATION OF HUMAN TUMOR ASSOCIATED ANTIGENS

Gold, D. V., University of Kentucky, School of Medicine, Dept. of Pathology, Limestone & Euclid, Lexington, Kentucky, 40506, United States of America

OBJECTIVES: Extract and purify colonic mucoprotein antigen (CMA) from normal colon and adenocarcinomas of colonic origin for immunologic and chemical analysis. Develop a radioimmunoassay and immunohistochemical detection procedures for CMA. Investigate the ability of antisera to differentiate between normal and tumor CMA to investigate the potential of CMA as a tissue specific or tumor specific marker. Investigate the oligosaccharide and peptide primary structure of CMA. Analyze the relationship of CMA to blood group substance, colon specific antigen, and carcinoembryonic antigen.

301. PURIFICATION OF TUMOR ANTIGENS OF DEFINED SPECIFICITIES

Gupta, R. K., University of California, Los Angeles Campus, School of Medicine, Dept. of Surgery, 2025 Zonal Ave., Los Angeles, California, 90024, United States of America

The specific aims of this proposal are to a) purify melanoma-associated antigens of defined specificities, b) develop radioimmunoassay (RIA) and enzyme immunoassay (EIA), c) apply these sensitive and specific serologic assays to detect antibody and free circulating antigen levels in sera and other body fluids of patients with malignant melanoma, and correlate the results of RIA and EIA with clinical course of disease. It is expected

that by developing the sensitive serologic assays and improving their specificities, these assays will become potentially useful tools for prognosis and diagnosis of human cancer. Cancer patients who are rendered disease-free by surgical removal of tumor can be monitored for the levels of free circulating tumor-associated antigens and their corresponding antibodies in the body fluids. Such information will be valuable to the physician for the management of human cancer. Besides being applicable as an immunodiagnostic aid, such information will expand the understanding and knowledge of tumor-host relationship. The isolation and purification of specific melanoma-associated antigens and their characterization will provide an insight into the immunobiology of human malignant melanoma.

302. STUDY OF THE NONSPECIFIC CROSS-REACTING ANTIGEN (NCA) AS A MARKER OF HUMAN MYELOID CELLS

Burtin, P., Flundrin, G., Charbord, P., Frenoy, N., Institut de Recherches sur le Cancer, Laboratory of Immunochimistry, B.P. 8, 94800 Villejuif, France

OBJECTIVE: To study the nonspecific cross-reacting antigen (NCA) as a marker of human myeloid cells.

APPROACH: Anti-NCA serum is used to stain cells of granulocytic and monocytic series in smears of peripheral white blood cells and bone marrow, as well as adherent monocytes prepared from peripheral blood, and colonies obtained by bone marrow culture. The same antiserum is used to assay NCA in the serum of patients with malignant blood diseases.

PROGRESS: NCA is a differentiation antigen of myeloid cells. In the granulocytic cell line, NCA appears initially in the promyelocyte stage. Its presence in leukemic myeloblasts and myelocytes is related to their differentiation. It is absent in leukemias with undifferentiated myeloblasts and is of no use in regard to the classification of these cases.

NCA is also found in monocytes-macrophages derived from blood or bone marrow, but not in leukemic monoblasts.

NCA is highly increased in the serum of patients with chronic myeloid leukemia, and decreased in cases of acute myeloid leukemia.

303. HUMAN TUMOR ANTIGENS - ANTIGENIC ANALYSIS OF HUMAN FETAL SPLEEN

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The objectives of these investigations are to determine the existence of fetal-specific human splenic antigens, to characterize these materials physicochemically and immunochemically and to determine if assays for these constituents have any relevance to human disease.

Rabbits were immunized with aqueous extracts of human spleen and the antisera raised were then tested with a variety of both fetal and adult human tissues. The studies were carried out both by the use of precipitin-analysis in agar gel and by immunofluorescence studies. The physicochemical characterization of the material has been initiated by gel sieve chromatography and polyacrylamide gel electrophoresis.

To date, four human fetal splenic antigen systems have been demonstrated. As expected, alpha-1-fetoprotein was detected, and this system put aside. Interesting observations have been made concerning fetal hemoglobin (HbF), and a radioimmunoassay for the gamma chain of HbF has been established and is presently being examined in a variety of human disease states. In addition, immunofluorescence studies have revealed at least one T lymphocyte and one lymphocyte marker which have not, to the best of our knowledge, been described to date.

G. OTHER STUDIES OF IMMUNOLOGICAL METHODS FOR DIAGNOSIS OF CANCER

304. NEW IMMUNOLOGIC APPROACHES TO LYMPHOID NEOPLASMS

Frenkel, E. P., Demian, S., Forman, J., Kettman, J. R., Shay, J. W., Sheehan, R. G., Sheehan, W. W., Smith, R. G., Uhr, J. W., Vitetta, E. S., University of Texas, Health Science Center at Dallas, Southwestern Medical School, Dept. of Internal Medicine, 5323 Harry Hines Blvd., Dallas, Texas, 75230, United States of America

These studies are directed toward the characterization of human lymphoid neoplasms, particularly those of the B cell type, in an attempt to develop a better definition of a clinically applicable taxonomy, exploit the biologic 'tumor' markers in an attempt to detect the minimal disease, and explore aspects of pathogenesis and alternative therapeutic approaches in these tumors. Antibody secreting cells making anti-tumor antibodies (anti-idiotypic antibodies) are being cloned and individual clones sought to develop a library of antisera to be used in the detection of different forms of B cell tumors in man. Exploiting the fluorescence-activated cell sorter, an 'immunologic fingerprint' of each tumor is now being carried out and a search for clinical correlates with the specific 'fingerprints' is in progress. The secreted products of these tumors and the responsiveness of tumors to mitogens and T cell signals are also being used for classification and in an attempt to apply these for the diagnosis of minimal disease. These studies propose a phenotypic characterization of the immunologic form of human lymphoid neoplasms that can be correlated with the classical histologic characterization of these tumors and provide criteria for therapeutic intervention.

BIBLIOGRAPHIC REFERENCES: Frenkel, E.P., and Kitchens, R.L. Acetyl-CoA synthetase from baker's yeast (*Saccharomyces cerevisiae*). Methods in Enzymology - Lipids, Part C. (ed., John Lowenstein). Academic Press, New York, 1980. Neuwelt, E.A., Maravilla, K.R., Frenkel, E.P., Barnett, P., Hill, S., and Moore, R.J. Use of enhanced computerized tomography to evaluate osmotic blood-brain barrier disruption. *Neurosurg.* 6:49-56, 1980.

305. PROGNOSTIC FACTORS IN NON-HODGKIN'S MALIGNANT LYMPHOMAS WITH EMPHASIS ON THE FUNCTIONAL APPROACH IN CLASSIFICATION OF LYMPHOMAS

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OBJECTIVE: Evaluation of prognostic factors in non-Hodgkin's lymphoma.

APPROACH: The project consists of two closely associated parts: a) A retrospective survival study of a defined Finnish case population of non-Hodgkin's lymphomata using the Lukes-Collins classification, Lennerts classification and, in addition, Rappaport classification. b) A prospective study: a versatile analysis of lymphoproliferative disorders by means of morphology, immunology, cytochemistry and cell kinetics.

The modern immunohistochemical techniques, the immunoperoxidase method for demonstration of intracytoplasmic immunoglobulins (large cell B-lymphomas) and murexase ('histiocytic lymphoma'), which can be performed on paraffin embedded material, are also applied to the study of the retrospective material.

306. RELATIONSHIP BETWEEN SURFACE MARKERS AND CYTOCHEMICAL STAINS IN ALL

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OBJECTIVE: To determine the relationship between these two methods of classifying acute lymphatic leukemia in children, with emphasis on determining the relationship between subpopulations of T leukemias and acid phosphatase stain.

METHOD: Upon diagnosis, studies are performed on membrane receptors using the rosette formation test (T lymphocytes) and the synthesis of immunoglobulins (B lymphocytes), with which the leukemia is classified into T-B-null.

Then studies are performed with cytochemical stains (PAS acid phosphatase), and clinical studies are performed (thoracic X-ray, etc.).

The evolution of the child is followed to observe the behavior of the various subgroups, with special focus on the 'null' acid phosphatase positive leukemias.

PROGRESS: This study has been underway for one year, during which time 35 children have been studied. It has been determined that 90% of the T leukemias are acid phosphatase positive. The significance of the other subgroups remains to be seen.

307. THE QUEEN ELIZABETH HOSPITAL LYMPHOMA-LEUKEMIA GROUP

Leong, A. S., Forbes, I. J., Sage, R. E., Liew, S. H., Dale, B., Queen Elizabeth Hospital, Dept. of Pathology, Woodville, S. Australia 5011, Australia

The Queen Elizabeth Hospital serves as a referral center for lymphoma-leukemia in the state of South Australia. Our research objective is to pursue investigation into the diagnosis and immunologic typing of malignant lymphoma, leukemia and related immunoproliferative disorders. The approach is multi-parameter, employing a battery of immunologic surface marker techniques, histochemistry, and ultrastructural and immunoperoxidase techniques for the identification and immunologic typing of the lymphoproliferation malignancies. An attempt is being made to correlate cell morphology with immunologic characteristics with the ultimate aim of recognizing consistent morphologic features which will correlate with the immunologic subclasses of lymphocytes. A number of other ancillary research projects related to clinical behavior and therapeutic responses of the various immunologic types of lymphomas and leukemia and the identification of new immunologic surface markers are also being conducted.

BIBLIOGRAPHIC REFERENCES: Leong, A. S.-Y., Forbes, I. J., Cowled, P.A., Sage, R. E., Black, R. B., Dale, B., and Zalewski, P. D.: Surface Marker Studies in Chronic Lymphocytic Leukemia and Non-Hodgkin's Lymphoma. *Pathology* 11:461-71, 1979. Forbes, I.J., Leong, A. S.-Y., Sage, R. E., Dale, B., and Cowled, P.A.: B Cell Leukemia Distinguished from Chronic Lymphocytic Leukemia by Surface Markers. *Aust. N. Z. J. Med.* 8:532-538, 1978.

308. IMMUNODIAGNOSIS IN LYMPHOPROLIFERATIVE DISORDERS

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OBJECTIVES: Definition of immunological parameters helpful in the diagnosis of the lymphoproliferative disorders.

APPROACH: Analysis of lymphocyte cell markers by immunofluorescence, cytotoxicity and immunoperoxidase and correlation with histopathological-cytochemical parameters.

309. CELL-MEDIATED IMMUNITY AND ASSAY FOR BREAST CANCER

Yonemoto, R. H., Chee, D. O., Nakamura, T., City of Hope Medical Center, 1500 E. Duarte Rd., Duarte, California, 91010, United States of America

The goal for the proposal is to develop an assay of cell-mediated immunity in breast cancer with clinical applicability, i.e., prognosis, guide to therapy and diagnosis. The rationale for the proposal is: 1) Improvement of leukocyte adherence inhibition assay, 2) utilization of highly purified TAA which should result in greater specificity in the leukocyte adherence inhibition assay (LAI), 3) parallel use of normal tissue antigen from same patient as control, 4) the use of sensitized frozen lymphocyte (allogeneic) because of easy accessibility of large quantities, access to repetitive determinations at convenience, and easy application of blocking assay, 5) in addition, basic understanding of the mode of action of LAI, i.e., cellular participation, lymphokine study, interplay of blocking factor, which will lead to an improved assay. To accomplish this goal, studies will be performed in two categories of patients.

A vertical study (patients to be entered during first two years) includes patients with benign and malignant disease, to seek comparative efficiency of LAI utilizing fresh PBL vs. frozen lymphocytes. A horizontal study (1st, 2nd and 3rd year) will evaluate patients at 1, 3 and more than 5 years post-mastectomy with no evidence of disease, as well as all patients at point of recurrent disease. All patients will undergo LAI and blocking assays at 3 month intervals as follow-up to therapy.

An improved CMI assay to monitor breast cancer patients will materialize at the end of 3 years.

BIBLIOGRAPHIC REFERENCES: Tanaka, Fumitaka, Yonemoto, Robert H., and Waldman, Stephen R. Blocking Factors in Sera of Breast Cancer Patients. *Cancer*, 43 (in press), 1979.

310. IMMUNOLOGICAL STUDIES IN BREAST CANCER

Baldwin, R. W., Robins, R. A., University of Nottingham, Cancer Research Campaign Lab., University Park, c/o City Hospital, Nottingham NG7 2RD, England, United Kingdom

OBJECTIVE: Development of immunological methods to aid detection, diagnosis, determination of progress and monitoring of human breast carcinoma.

APPROACH: Measurement of serum immune complexes has shown elevated levels of C1q binding activity in the serum of breast carcinoma patients. However, this approach has proved insufficiently sensitive and specific for the objective outlined above. Monoclonal antibodies are therefore being raised by immunization of mice with primary breast carcinoma derived cells. Antibody preparations will be used for flow cytofluorimetric analysis of primary tumor derived cells, and will be used to detect tumor derived products in the circulation.

PROGRESS: Although a number of breast carcinoma cell lines have been established, the prolonged growth of human malignant breast cells in culture is comparatively rare. It is likely, therefore, that established cell lines are not typical of breast carcinoma in general. We have therefore attempted to raise monoclonal antibodies to primary breast carcinoma cells. The feasibility of using cells derived from primary carcinoma for immunization of mice and screening of hybridoma products has been established. Several antibodies reacting with carcinoma derived cells but not lymphoid cells from the same patient are currently being evaluated.

311. IMMUNODIAGNOSIS OF PROSTATIC CANCER

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The objective of this proposed study is to develop and evaluate the immunodiagnostic method for an early detection of prostatic cancer. Prostatic acid phosphatase and ribonuclease are to be investigated in depth. These enzymes will be purified to homogeneity from human cancerous prostatic tissues and prostatic fluids with established methodologies. Specific immune sera are then raised with the purified enzymes in small animals (rabbits or goats). The sensitive immunochemical techniques (solid-phase fluorescent non-isotopic immunoassay for acid phosphatase, radioimmunoassay and enzyme-linked immunoassay for ribonuclease) will be developed utilizing the purified enzymes and their specific antiserum for measurements of minute amounts of enzymes in serum. Patients with cancer of the prostate and other tumors as well as controls will be evaluated. The ultimate goal of this proposal is to detect, by means of immunodiagnosis, the early, localized and treatable prostatic tumor.

BIBLIOGRAPHIC REFERENCES: Lee, C.L., Berjian, R., Sarcione, E. and Chu, T.M.: Ribonuclease isoenzymes purified from ascites fluid of human pancreatic cancer. *Fed. Proc.* 39:774, 1980 (abstract). Lee, C.L., Killian, C.S., Murphy, G.P. and Chu, T.M.: A solid-phase immunoabsorbent assay for serum prostatic acid phosphatase. *Clin. Chim. Acta* (in press, 1980).

312. IMMUNODIAGNOSTIC MEASURES IN CERVICAL CANCER

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The objective of this study is to systematically assess the reliability and validity of the AG-4 Serological Test in predicting the subsequent course of cervical cancer. This tumor-specific antigen has been shown to be present in most of the patients with invasive cervical cancer or with recurrent cervical cancer, to be virtually absent in controls without cervical disease and to be present in a minority of patients with pre-neoplastic cervical disease. Patients in various stages of cervical neoplasia are being followed prospectively in order to measure their blood levels of AG-4 before, during and after invasion.

313. BIOCHEMICAL AND IMMUNOLOGICAL MARKERS OF DIAGNOSTIC AND PROGNOSTIC IMPORTANCE IN ORAL CANCER

Shirodkar, S. R., Ghanekar, S. N., Vaidya, S. G., Harinath, B. C., G.M. Hospital & Research Inst., Laboratories of Biochemistry

The summary for this project is under entry no. 40.

314. IMMUNOLOGIC STUDY OF CANCER OF THE COLON AND RECTUM

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Cancer of the colon, due to its antigenic properties, is one of the most interesting sites for immunologic study. This is reflected in available bibliographic references to the use of CEA, biological and enzymatic markers, and other tumor-associated antigens.

Several years ago, we began to study the role of immunology in patients with digestive cancers through use of the DNCB test with a challenge dose of 50 or 100 micrograms. A negative reaction is classified as immunosuppression. A slight positive reaction or small erythema at one or two sites is classified as immunodepression. A positive reaction of 2 plus or 3 plus erythema with hardness is classified as normal immunity, and 4 plus in cases with large phlyctenae is classified as subnormal.

In gastric cancer, there is a relationship between immunologic state and prognosis. In cancer of the colon and rectum, we have analyzed the relationship of immunity to the degree of tumoral invasion according to the Duke's classification and survival. In 150 patients studied, we observed the following: Grade A: 2 plus 50 percent; and 3 plus 50 percent. Grade B: negative 57 percent, 1 plus 18 percent, 2 plus 14 percent, 3 plus 5 percent, and 4 plus 6 percent. Grade C1: negative 43 percent, 1 plus 26 percent, 2 plus 23 percent, 3 plus 3 percent, and 4 plus 3 percent. Grade C2: negative 60 percent, 1 plus 10 percent, 2 plus 25 percent, and 4 plus 3.5 percent.

Among those patients under study without adjuvant chemotherapy or immunotherapy that might alter the immunologic status and therefore the prognosis, immunosuppressed patients died within 2-12 months. Immunodepressed patients died within 8-12 months. Of normal and subnormal patients in the first series, 1/8 died at 2 years; and 1/8 died from the second series.

The purpose of this study is to analyze the groups of patients who were not treated, comparing them with patients receiving chemotherapy and immunotherapy to determine the alterations in immunity using the DNCB tests. (Translated)

315. DETECTION AND DIAGNOSIS OF COLORECTAL CANCER

Baldwin, R. W., Hardcastle, J. D., University of Nottingham, Cancer Research Campaign Lab., Dept. of Surgery, University Park, c/o City Hospital, Nottingham NG7 2RD, England, United Kingdom

OBJECTIVE: The development of immunological tests for early detection and diagnosis of colorectal cancer.

APPROACH: Screening for colorectal cancer is being investigated using a range of immunological assays for detecting tumor-related products in serum, other body fluids and faeces. Tests for the determination of occult blood in feces are being developed and their use is being evaluated in patients with symptoms suggestive of colonic or rectal malignant disease. Tumor-related products are also being monitored in serum and colonic washings pre-and post-operatively in colorectal cancer for monitoring treatment.

PROGRESS: Colorectal Cancer Screening - An immunological assay has been developed for detecting hemoglobin in feces; this assay discriminates human from animal hemoglobins (Lancet 8210,18, 1981). This assay, together with commercial hemoglobin tests, is being evaluated in patients known to have malignant or benign colorectal tumors and in patients presenting to general practitioners with abdominal symptoms.

DIAGNOSIS - Serum immune complex levels (CIC) have been determined using a modified (C1q) binding test in pre-operative patients with colorectal cancer. CIC levels were elevated in 61% of patients, this not being related to stage of disease or tumor mass. Serum C1q binding was also elevated in patients with benign polyps (Brit. J. Surgery, in press)

316. IMMUNODIAGNOSIS OF PANCREATIC CANCER

Douglass, H. O., Goldrosen, M. H., Roswell Park Memorial Inst., 666 Elm St., Buffalo, New York, 14263, United States of America

Leukocytes from a total of 212 patients have been assayed against an antigen derived from pancreatic adenocarcinoma. Results, to date, have indicated that the leukocytes from 11 of 12 patients whose cancers are localized or confined to lymph nodes adjacent to the pancreas have identified the antigen, whereas leukocytes in 6 of 9 patients with more distant lymph node involvement have identified that antigen. Among patients with metastatic pancreatic carcinoma with either intra-abdominal spread or hematogenous spread to organs other than the liver, leukocytes from 10 of 12 patients have identified the antigen. In contrast, leukocytes from only 15 of 27 patients with metastatic pancreatic adenocarcinoma of the liver have identified these antigens. There have been no false positives among the 17 patients with pancreatitis, 2 false positives among 16 patients with other diseases of the gastrointestinal tract and one false positive among 30 normal healthy controls. Among patients with carcinoma, there have been 2 false positives in the group of 71 patients with gastrointestinal tract cancer (both had cholangiocarcinoma) and 2 other false positives in the group of 18 patients with carcinomas of other than gastrointestinal origin. Preliminary studies evaluating serum 'arming' of normal healthy leukocytes and the use of those 'armed' leukocytes in LAI have shown concordant results in 76% of tests thus far. Further studies are under way to assess the value of 'arming' and seek standardization of an antigen source.

BIBLIOGRAPHIC REFERENCES: Goldrosen, M.H., Russo, A.J., Howell, J.H., Leveson, S.H., Moore, M.C., Holyoke, E.D., and Douglass, H.O. Jr. Micro-Leukocyte Adherence Inhibition Evaluation as an Immunodiagnostic Test for Pancreatic Cancer. Cancer Res. 39 (February 1979).

317. IMMUNODIAGNOSIS OF MESOTHELIOMA

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Mesotheliomas, malignant tumors of the serous lining of the body cavities, are notoriously difficult to differentiate from other tumors involving these cavities. An antiserum specific for mesothelial cells was developed by immunizing rabbits with cultured mesothelial cells and appropriate absorptions. The antibody has been used in indirect immunofluorescence and immunoperoxidase staining assays to differentiate mesotheliomas from other malignant tumors.

The antibody described above was of low titer and could not be used to isolate mesothelial cell specific antigen by affinity chromatography. A large batch of cultured normal mesothelial cells will be extracted with PbS containing 0.1% NP 40 and subjected to negative affinity chromatography with antisera to fetal calf serum, human serum, and colon and breast carcinoma tissues. The partly purified mesothelial antigen will be used to raise a batch of high titered antibody to mesothelial cells. The antibody will be used to isolate the mesothelial antigen. The high titered antibody and the isolated pure mesothelial cell antigens will then be employed to develop a quantitative immunoassay for mesothelial antigen. The assay will be used as a non-invasive diagnostic tool for mesotheliomas as well as for quantitation of mesothelial cell mass.

318. IMMUNOFLUORESCENT IDENTIFICATION OF BASEMENT MEMBRANES

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OBJECTIVE: Perform indirect immunofluorescent localization of reactive structures with specific antibodies, utilizing antibodies against: purified tumor basement membrane collagen; type I procollagen; type III procollagen; and fibrinogen or some other noncollagenous, nonconnective tissue protein.

319. THE LEUKOCYTE ADHERENCE INHIBITION TEST - A STUDY OF THE CLINICAL USES AND MECHANISM OF THE TEST

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We propose to evaluate the leukocyte adherence inhibition (LAI) test with respect to clinical usefulness in diagnosing possible breast and lung tumor patients and monitoring their progress during and after treatment. The technique used will be a modification of the tube LAI test. Procedures will be used to detect

the presence of monocyte 'arming' cytophilic antibody in the serum of suspected tumor patients and individuals being treated for tumors. The monocyte chemotaxis inhibition procedure will also be used in our clinical evaluations to provide a basis for comparison of the effectiveness of the LAI test.

Attempts will be made to provide data that will clarify the mechanism of the LAI test. We propose to vary conditions of the test to determine whether different assay procedures result in changes of the cell types and mechanism of the test. We postulate that under certain assay conditions, a T-cell mediated response will be detected while other conditions will result in the detection of a monocyte-cytophilic antibody response.

An attempt will be made to purify and partially characterize the LAI-reactive material in breast and lung tumors. Purification will be attempted using differential centrifugation, gel filtration chromatography, and affinity chromatography. The LAI-reactive molecule(s) will be characterized with respect to sensitivity to a variety of physical, chemical and enzymatic agents, molecular weight, and electrophoretic and ultracentrifugation characteristics.

320. LEUKOCYTE ADHERENCE INHIBITION ASSAY IN HUMAN CANCER

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During fiscal 1980-81, it is proposed to continue evaluation of leukocyte adherence inhibition (LAI) analysis as a means for detection and diagnosis of cancer in man. Enough data should be available to begin preliminary evaluation using the matrix design originally described. In addition, comparisons with non-hemacytometer variants of the LAI system will be made.

The availability of an image analyzer will permit experiments designed to quantify the test. The basic experiments will have been done by the end of the above fiscal year.

Finally, experiments will be done to establish the role of HLA genetic restriction to tumor antigen presentation in elicitation of the LAI response. This will be done by means of analyses of HLA-typed families of tumor patients and by the use of specific anti-HLA sera. Preliminary experiments using as a model immunization of humans to keyhole limpet hemocyanin have revealed the likely existence of such a restraint.

321. EVALUATION OF A MICRO-LEUKOCYTE ADHERENCE INHIBITION ASSAY AS AN IMMUNODIAGNOSTIC TEST FOR GASTROINTESTINAL CANCER

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The primary objective of this proposal is to evaluate the potential utility of the leukocyte adherence inhibition (LAI) assay as an immunodiagnostic test for gastrointestinal cancer. This assay will be applied to new patients who are referred to a gastrointestinal out-patient clinic for diagnostic evaluation at the Veterans Administration Hospital, Buffalo, New York, a university affiliated teaching hospital of the State University of New York at Buffalo. Patients with a gastrointestinal (colorectal, pancreatic, gastric) malignancy, occult symptoms suggestive of a possible gastrointestinal malignancy or with a disease that has malignant potential will be chosen for this study. Eventual clinical diagnosis will indicate that 20% of this population will have malignant gastrointestinal disease, while the balance of these patients will have benign gastrointestinal disease or are normal. Isolated leukocytes of coded specimens will be evaluated in the microplate LAI assay against pretested biologically active tumor extracts of colon, gastric and pancreatic tumors. Correlations between test results and the patients clinical status will be performed to determine if this assay is a sensitive, reproducible and practical test that can detect specific forms of localized gastrointestinal cancer and differentiate them from benign gastrointestinal disease and the normal state.

322. IMMUNOPREVENTION OF LUNG CANCER UTILIZING THE LEUKOCYTE ADHERENCE INHIBITION TECHNIQUE

Morini, J. C., Habegger de Sorrentino, A. M., Rousseau, V. H., Dellamea, R. O., Pascual, D., Sorrentino, A., Coca, A., Hospital

Julio C. Perrando, Laboratorio de Immunologia, Avenida 9 de Julio 1101, Resistencia, Chaco, Argentina

OBJECTIVE: The importance of this method lies fundamentally in the possibility of early immunologic detection of lung tumors, thereby allowing a more immediate treatment of patients.

APPROACH: In a group of patients over 45 years of age who smoke, have chronic cough and repetitive broncho-pulmonary pathology, and are likely to develop neoplasias, detection of cancer by the leukocyte adherence inhibition (LAI) technique will be studied. LAI consists of the following: leukocytes have the property of spontaneously adhering to test tube walls. Sensitization of these leukocytes with an antigen causes the adherence to diminish. This technique will be used employing antigens specific to lung tumors, in three groups of patients: Group A - patients at high risk (age 45, smokers, chronic cough with repetitive broncho-pulmonary pathology). Group B - Patients with benign lung pathology. Group C - Normal controls. Among these groups, patients will be matched on age and sex.

The LAI technique of Holliday and colleagues will be used: 0.05 ml leukocytes from patients will be challenged with 0.05 ml of the following antigens: Lung tumor extract antigen consisting of a pool of squamous cell carcinoma, adenocarcinoma and small cell carcinoma; normal tissue antigen; P.P.D. antigen. The various antigens are diluted 1/5 and each tube is completed with 0.2 ml Hank's tissue culture solution. One tube contains only the leukocytes and Hank's solution in order to determine the normal adherence. An initial leukocyte count is made for all tubes which are then incubated lying flat and vigorously agitated every 5 minutes for 30 minutes. A final leukocyte count is made of the supernatant fluid only; and by comparison with the first count, the percentage of adherence is calculated.

323. APPLICATION OF IMMUNOLOGICAL METHODS FOR CHARACTERIZATION OF HUMAN NEOPLASMS

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OBJECTIVE: Immunohistochemical characterization of neoplastic cells.

APPROACH: Immunoperoxidase techniques both at the light and electron microscopic level. Several different types of tumors are investigated: 1. APUD tumors with regard to the presence of insulin, glucagon, gastrin, CCK, secretin, ACTH, calcitonin, somatostatin, VIP and pancreatic polypeptide; 2. germ cell tumors with regard to the presence of alpha-fetoprotein and chorionic gonadotropin; 3. cystic ovarian teratomas for the presence of APUD cells. In general the presence of peptides in neoplastic cells is established and related to histological features. Attempts are made to extract new criteria for diagnosis, prognosis and treatment.

324. IMMUNOLOGICAL REACTIONS IN CANCER OF MAN AND ANIMALS

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OBJECTIVE: To study the leukocyte adherence inhibition test in man and experimental animals with regard to its mechanisms, specificity, and modification by serum factors, and to apply the test in immunological diagnosis and monitoring of human cancer.

APPROACH: The characteristics of the LAI test have been worked out with chemically induced tumors of mice, and shown to apply to natural tumors of men. It was anticipated that all tumors would have characteristic antigens, to which their hosts would respond consistently, and that these responses would be detectable in vitro.

PROGRESS: The LAI test has been shown to depend on the reaction between soluble tumor antigens and T-lymphocytes, with the liberation of a lymphokine (within 20 minutes) which diminishes the normal adherence to glass of macrophages or polymorphs. The kinetics of development of cell-mediated immunity to tumor antigens have been studied in mice, as have the phenomena of blocking and unblocking by serum factors. These parameters are determined with great regularity in human patients, notably those with melanoma, colon carcinoma and

medullary carcinoma of thyroid. In human cancer the LAI specificity is related to tumor type. Well-defined patterns of reactivity are found in all stages of disease.

325. IMMUNOLOGICAL CRITERIA IN THE DETECTION OF HIGH OCCUPATIONAL CANCER RISK AMONG TEXTILE WORKERS

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The purpose is to examine textile workers handling direct dyes containing benzidine and beta-naphthylamine. The workers have been divided into 3 groups according to extent and duration of exposure. The following immunological tests have been used: (1) determination of benzidine as haptene in the blood serum of workers, splitting of the direct dyes by specific rabbit immune serum; (2) determination of an endogenous carcinogen, the metabolite of tryptophane-3-hydroxyanthranilic acid (3HAA), using specific rabbit immune serum in immune diffusion in agarose as a criterion of disturbances in tryptophane metabolism. Such disturbances have frequently been observed in patients with cancer of the urinary bladder and some other sites (breast, lung); (3) determination of the index of phagocytic activity of blood neutrophils as related to specific and non-specific factors. Particles of activated carbon, loaded by benzidine- and 3-HAA -azoproteins, were used as specific factors, and staphylococcus aureus as a non-specific factor; (4) determination of blast transformation of peripheral leucocytes to PHA and benzidine- and 3-HAA-azoproteins. The blood of non-exposed workers and healthy donors was used as a control.

326. PHAGOCYTE FUNCTION IN CANCER AND ANTICANCER THERAPY

Solberg, C. O., Haukeland Sykehaus, Dept. of Medicine, N5016 Bergen, Norway

OBJECTIVES: 1) To examine whether granulocyte and monocyte function studies are superior to morphological methods (peripheral blood smears and bone marrow aspirates) in the early diagnosis of myelomatosis and acute leukemia i.e., preleukemia. 2) To study the influence of anticancer therapy on granulocyte and monocyte functions.

APPROACH: 1) Granulocytes and monocytes are isolated from the peripheral blood of patients who might be in the early state of myelomatosis or acute leukemia, i.e., patients with enhanced susceptibility to infection and/or anemia, thrombocytopenia or increased erythrocyte sedimentation rate of unknown origin. Oponized bacteria are added to the cell suspensions and the phagocytic and bactericidal activities are measured. In addition, leukocyte chemiluminescence, adhesiveness (by glass bead columns) and in vivo and in vitro chemotaxis are measured. 2) The influence of anti-cancer drugs on leukocyte functions is examined using the same techniques as described above.

PROGRESS: 1) In some patients, impaired bactericidal activity of the leukocytes has been demonstrated from 1 to 14 months before leukemia could be diagnosed from clinical and hematological findings. *Scand. J. Infect. Dis.* 8, 53-55, 1976. Since the preleukemic stage is considered the most favorable stage for onset of antileukemic therapy, phagocyte function studies might contribute to the improvement of therapeutic results. An improved skin window technique has been developed for the in vivo measurement of leukocyte migration. Impaired migration has been demonstrated in acute leukemia. *Acta Path. Microbiol. Scand. Section C* 85, 413-423, 1977. 2) Phenylbutazone reduces leukocyte glucose metabolism and function. *J. Reticuloendothel. Soc.* 20, 447-455, 1976. Corticosteroids significantly impair leukocyte phagocytosis and glucose metabolism, and to a minor degree intracellular killing of bacteria. *Acta Path. Microbiol. Scand. Section C* 85, 284-288, 1977.

327. EYE TUMORS - IN VITRO IMMUNODIAGNOSIS AND MONITORING

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Immunologic and cell culture techniques will be used to further our understanding in three areas of ocular oncology.

Retinoblastoma may occur as a sporadic or germinal mutation; 10% of germinal mutation retinoblastoma survivors develop second malignancies. Genetic mechanisms are important in the development of retinoblastoma; this eye tumor may serve as an important model for inherited malignancies. The differentiation of sporadic from hereditary (germinal mutation) retinoblastoma patients may be difficult. Preliminary data demonstrates that hereditary retinoblastoma patients' fibroblasts are more sensitive to ionizing radiation than fibroblasts from sporadic retinoblastoma patients or normal controls. We will use a number of mutagenic techniques to attempt to better delineate the nature of the defects in DNA repair mechanisms in retinoblastoma. These studies may allow us to better differentiate hereditary from sporadic retinoblastoma patients, increase our knowledge regarding DNA repair defects in retinoblastoma, and predict which patients are more likely to develop second malignancies.

Patients enucleated with large choroidal melanomas have approximately 50% five year tumor-related mortality. Some investigators believe that enucleation may increase mortality secondary to increased tumor cell dissemination. If this hypothesis is true, pre-operative irradiation may decrease tumor mortality. We will test, using in vitro and in vivo cell culture techniques, whether adjunct radiation will decrease the tumor cell viability and metastatic potential of these choroidal melanoma cells.

The histologic differentiation of orbital pseudotumor from lymphoma has an error rate approaching 50%. We wish to use immunologic surface marker studies to better differentiate these orbital tumors which have a marked difference in prognosis. Similar technology will also be used to study the nature of lymphocyte infiltrations in uveitis and ocular reticulum cell sarcoma. It is possible that these techniques may allow us to increase both our diagnostic accuracy and basic understanding of lymphatic infiltration of ocular structures.

III. CYTOLOGICAL STUDIES IN CANCER DIAGNOSIS AND PROGNOSIS

A. CYTOGENETIC STUDIES FOR CANCER DIAGNOSIS AND PROGNOSIS

328. CHROMOSOME MARKERS AND NON-RANDOM KARYOTYPE REARRANGEMENTS IN MYELO- AND LYMPHOPROLIFERATIVE DISORDERS

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OBJECTIVE: Non-random karyotypic abnormalities have been shown in most of myelo- and lymphoproliferative disorders. Aim of this study is a more extensive investigation of the occurrence of specific chromosomal abnormalities in peculiar disorders such as acute monoclonal and acute promyelocytic leukemias, as well as in some conditions of neoplastic lymphoid proliferation (plasma cell leukemia, myelomatous effusions, acute leukemia associated with plasmacytoma).

APPROACH: Conventional banding techniques (Q, G, C, R) have been applied to chromosome preparations from bone marrow and effusions obtained by direct method. Short-term cultures (up to 18 h) without addition of PHA have been also utilized. For the analysis of the markers involving satellited chromosomes (D, G), techniques for demonstration of NOR's as well as of persisted nucleolar remnants have been employed.

PROGRESS: Nine monoclonal and five promyelocytic leukemias have been observed among 82 consecutive acute non-lymphoid leukemias. No consistent chromosome patterns have been demonstrated in monoclonal leukemias. Two patients with promyelocytic leukemia exhibited a typical translocation t(15;17) in addition to other abnormalities (one patient with microgranular variant). Structural rearrangements of chromosomes No. 1 and No. 14 were most commonly observed in myelomatous effusions. Frequency of the association of abnormal No. 14 (14 q plus sign) with nucleolar remnants identified cytochemically is currently under investigation.

329. STUDY OF LEUKEMIA AND PRE-LEUKEMIC STATES USING BONE MARROW COLONY FORMATION AND CHROMOSOMAL BANDING

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Bone marrow and peripheral blood colony formation was studied in patients with acute leukemias and chronic myeloproliferative disorders. Peripheral blood colony formation was markedly elevated in three patients with the diagnosis of chronic myelogenous leukemia, whereas it was normal in a patient with polycythemia rubra vera. Bone marrow colony formation in one patient with chronic myelogenous leukemia was markedly increased. Bone marrow colony formation in a patient with a hypercellular acute myelomonocytic leukemia was low, whereas another patient with 20% absolute marrow myeloblast percentage had low normal marrow colony formation but 13-fold increase in peripheral blood colony formation. An additional unusual patient had a severe peripheral blood pancytopenia and hypercellular oligoblastic marrow for one year, followed by a marked peripheral blood neutrophilic leukocytosis resembling chronic myelogenous leukemia, with a 25-fold increase in marrow colony formation and 250-fold increase in peripheral blood colony formation; however, no Philadelphia chromosome was present. One patient with acute lymphocytic leukemia, who was in peripheral blood and bone marrow remission, had significantly reduced bone marrow colony formation two months prior to bone marrow relapse. These studies will be continued with the goal of identifying patterns which may allow for prediction of response to therapy.

330. THE CLINICAL APPLICATION OF CHROMOSOME ANALYSIS TO THE DIAGNOSIS, PROGNOSIS AND TREATMENT OF LEUKEMIA AND CANCER

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OBJECTIVE: Our basic objective is to study the clinical application of chromosome analysis in leukemia and related disorders. We hope to encourage the development of additional cytogenetic laboratories in other hospitals and to provide this service to small hospitals which cannot establish a cytogenetic laboratory.

APPROACH: A) We are developing improvements in techniques which will shorten the time required for analysis of chromosomes from bone marrow aspirates. B) We are compiling a file of abstracts published on chromosome studies in human leukemia, preleukemia, related hematologic disorders, lymphoma and solid tumors. These abstracts are catalogued and cross-indexed under approximately 250 index terms, and the files are organized for rapid retrieval. C) Educational material will be distributed to designated hospitals and physicians.

PROGRESS: We have developed a procedure of collecting bone marrow aspirates in EDTA with subsequent refrigeration which maintains the suitability of the specimen for chromosome analysis for 24 hours. This eliminates the urgency of processing bone marrow specimens immediately and allows the processing to be scheduled once daily. This procedure also allows mailing of bone marrow aspirates within the continental United States.

331. EARLY DETECTION AND MONITORING OF LEUKEMIA AND TUMORS IN CHILDREN AND ADULTS

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OBJECTIVE: 1) Recording of so-called preleukemic states, i.e. hematological disorders highly prone to develop into leukemia. 2) Early detection of malignant lymphoma in children and adults. 3) Recording of mutagenic effects of the cytostatic therapy. 4) Cytogenetic characterization of solid tumor cells.

APPROACH: Chromosomally abnormal (supposed to be malignant) cells are to be detected and recorded by a systematic cytogenetic analysis which is already being carried out in so-called preleukemic states. As for malignant lymphoma, special emphasis is placed on investigating the cell population which is supposed to be the subject of malignant transformation by using

appropriate techniques of cell isolation, stimulation and culture. In tumor patients also showing signs of known DNA-repair defects, the ability of their cells to repair experimentally induced damage by UV-light, X-rays or chemicals is tested using cytogenetics and biochemical methods. In order to record possible mutagenic effects of tumor therapy chromosome defects, rate of sister-chromatid exchange and aberration in the DNA content of somatic and sperm cells are being searched for in different stages of the disease.

PROGRESS: In 1980 cells of over 400 patients belonging to one of the above groups could be investigated. We have also started to collect data systematically on the patients' personal and family histories by means of questionnaires and interviews designed to gain insight into the etiology of the disorders. This information is now being taken into consideration in evaluating the laboratory results. Some aspects of this study have been singled out for publication.

332. CYTOGENETIC STUDIES OF ACUTE LYMPHOBLASTIC LEUKEMIA IN CHILDHOOD

Lawler, S. D., Hardisty, R. M., Secker-Walker, L. M., Royal Marsden Hospital, Dept. of Cytogenetics & Immunology, Fulham Rd., London SW3 6JJ, England, United Kingdom

OBJECTIVE: Cytogenetic studies of acute lymphoblastic leukemia in childhood.

APPROACH: Detailed cytogenetic follow-up of a cohort of children with acute lymphoblastic leukemia at diagnosis, throughout treatment and in relapse.

BIBLIOGRAPHIC REFERENCES: Acute Myeloid Leukemia with Monosomy-7 Follows Acute Lymphoblastic Leukemia. Secker-Walker, L.M. and Sandier, R.M. *B.J. Haemat.* 1978, 38, 359-366. Prognostic Implications of Chromosomal Findings in Acute Lymphoblastic Leukemia at Diagnosis. Secker-Walker, L.M., Lawler, S.D. and Hardisty, R.M. *B. Med. J.* 1978, 2, 1529-30. Ph⁺ Positive Leukemia in Childhood. Chessells, J.M., Janossy, G., Lawler, S.D. and Secker-Walker, L.M. *B. J. Haemat.* 1979, 41, 25-42.

333. STUDY OF CYTOGENETICS IN CARCINOMA OF THE CERVIX UTERI

Gupta, S., Sanyal, B., Singh, I. J., Gupta, I. M., Bomjai, R., Gupta, Y. N., Banaras Hindu University, Inst. of Medical Sciences, Dept. of Pathology, Varanasi 221005, Uttar Pradesh, India

OBJECTIVE: It is believed that cells of the cervical epithelium undergo Euploidy when it passes from normalcy to the stage of dysplasia and finally becomes cancerous. The purpose of the study is to do the Karyotyping of cells of the cervix carcinoma and find biological markers if any and determine the relationship of Euploidy (especially of Tetraploidy) to biological behavior of the tumor.

APPROACH: Cancerous tissue from the cervix has been collected in tissue culture fluid and short-term cultures are grown. Squash preparations are also used. Separation of chromosomes is done by standard techniques. G. Barding is done wherever necessary.

334. CHROMOSOMES OF HUMAN BLADDER TUMORS

Sandberg, A. A., Matsui, S., Pontes, E. J., Wake, N., Roswell Park Memorial Inst., Dept. of Genetics/Endocrinology, 666 Elm St., Buffalo, New York, 14263, United States of America

This research project has addressed itself to a number of facets related to chromosome changes in bladder cancer. A major aim of the project was to define chromosomal changes in papillary (noninvasive) bladder tumors, with the hope of establishing parameters useful in predicting recurrence. To a large extent, this was accomplished through the demonstration that the presence of abnormal chromosomes (markers) results in more than 90 percent chance of recurrence of such tumors, whereas the absence of such markers has the opposite indication. More recently, we have been engaged in the development of a method for analyzing metaphases in bladder cancer so that the yield of such metaphases would be higher than usually obtained with presently available methods, result in preparations which are more suitable for banding and, thus, for more detailed analysis of some of the more complicated changes and the ability to determine the chromosome constitution on small pieces of tissue. This we think we have accomplished by utilizing collagenase II or IV and DNase I in disaggregating cells, followed by short-term incubation. At present, we are analyzing

as many of bladder cancers as become available with the hope of ascertaining nonrandom changes, as well as correlating the karyotypic findings with the clinical, therapeutic and prognostic parameters of the cases.

A study which addressed itself to centromeric heterochromatin polymorphism has about been completed and indicates that there appears to be no significant differences between the incidence of such polymorphism in the cells of patients with bladder cancer as compared to a control population of subjects without a history of cancer and 85 years old or older. These findings are of importance, since a number of laboratories have indicated a higher susceptibility to bladder cancer as indicated by a higher incidence of polymorphism in patients with this malignancy. Thus, more studies of a more refined nature will have to be undertaken in order to settle this issue.

Pilot studies are being performed in which bladder tumor samples are shipped to Buffalo for karyotypic analysis in order to ascertain the feasibility of undertaking such a study nationwide.

BIBLIOGRAPHIC REFERENCES: Sandberg, A.A. and Wake, N.: Chromosomal changes in primary and metastatic tumors and in lymphoma: Their nonrandomness and significance. In: *Genes, Chromosomes, and Neoplasia*, Arrighi, F.A., Rao, P.N. eds., Stubblefield, E., Raven Press, New York, pp. 297-333, 1980.

335. IDENTIFICATION OF COLORECTAL CANCER RISK

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The purpose of this research is to develop an in vitro assay system for detection of genetic predisposition for cancer of the colon and rectum.

Cell cultures from skin and colonic mucosa biopsies will be established from patients with colonic cancer with and without polyposis, their relatives at-risk and family members by-marriage and non-family members without a family cancer history as controls.

The influence of cancer-prone germinal mutations on the in vitro biological features (morphology, growth parameters, mitosis, surface topography and epithelial-specific enzyme activities) of cultures derived from these 4 groups will be evaluated.

Somatic mutations (spontaneous and induced by either the mutagen ethylmethane-sulfonate or ultraviolet light will be studied using the in vitro markers for 2 mutants, resistance to ouabain and ability to use fructose.

Transformation, defined as the acquisition of a biological property not present in the parent culture, (spontaneous and induced by either the carcinogen urethane or MNNG) will be studied by a panel of 11 properties considered to be criteria of transformation.

The aims of this in vitro research on colorectal cancer is to (1) delineate the biological properties of cancer of the colon and rectum and (2) to develop an assay to identify individual genetic risk of colorectal cancer.

336. KARYOTYPIC ANALYSIS IN RELATION TO CANCER SUSCEPTIBILITY, ETIOLOGY AND THERAPY

Watt, J. L., Brown, T., University of Aberdeen, Cytogenetics Lab., Dept. of Genetics, Foresterhill, Medical School Bldgs., Aberdeen AB9 2ZD, Scotland, United Kingdom

OBJECTIVE: The project is designed to evaluate the use of modern cytogenetic parameters in measuring susceptibility to cancer by comparing patients with confirmed double primary cancer with both 'single cancer' and 'asymptomatic' controls. A further aim of the project is to monitor patients with Hodgkin's disease undergoing modern radiotherapy/chemotherapy in an attempt to detect those at greatest risk of developing a treatment-induced, second malignancy. It is hoped that the study will aid early diagnosis of specific iatrogenic neoplasms and perhaps even provide a means of estimating predisposition to cancer in general, thus granting time for evasive action.

APPROACH: Chromosomes from blood and/or bone marrow cultures will be examined. Prometaphase karyotypes will be analyzed by G, and sequential C-banding and the rate of sister chromatid exchange will be estimated using a harlequin staining method. Photographic records of all chromosome analysis will be kept.

337. CHROMOSOMAL STUDY OF PATIENTS WITH NEUROFIBROSARCOMA IN VON RECKLINGHAUSEN'S DISEASE

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OBJECTIVE: Von Recklinghausen's disease is a congenital entity in which neurofibromas have a high probability of malignant degeneration. Chromosomal mapping of patients will be performed to look for the possibility of specific genetic alterations.

MATERIAL AND METHODS: All patients with confirmed diagnosis of neurofibrosarcoma in von Recklinghausen's disease and who display the symptoms of this disease will be studied. (Translated)

338. STUDIES OF DNA REPAIR IN HUMAN DEGENERATIVE DISEASES

Robbins, J. H., Moshell, A. N., Scarpinato, R. G., Snowden, K. I., Potkay, S., Tarone, R. E., Doniger, J., Scudiero, D. A., Lytle, C. D., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Dermatology Branch, 9000 Rockville Pike, Bethesda, Maryland, 20205, United States of America

Studies in this laboratory are designed to elucidate the role of DNA repair processes in human diseases and in carcinogenesis, mutagenesis, and normal and abnormal aging. Most studies have been conducted with cells from patients with xeroderma pigmentosum (XP), all of whom have defective DNA repair plus the clinical abnormalities of multiple cutaneous malignancies and premature aging of sun-exposed skin. Studies are currently being performed on cells from patients with degenerative neurological diseases such as Huntington's disease, ataxia telangiectasia, familial dysautonomia, olivopontocerebellar atrophy, and Cockayne's syndrome. These studies are designed to elucidate the pathogenesis of these disorders as well as to develop presymptomatic (including prenatal) diagnostic tests. We assess the biological effectiveness of DNA repair primarily by in vitro assays of post-irradiation colony-forming ability and trypan blue exclusion. We are extending our work to include cells from patients with other degenerative disorders including Shy-Drager syndrome, idiopathic orthostatic hypotension, retinitis pigmentosa, Usher's syndrome, spinal muscular atrophy, and muscular dystrophy.

BIBLIOGRAPHIC REFERENCES: Moshell, A.N., Tarone, R.E., Barrett, S.F., and Robbins, J.H.: Radiosensitivity in Huntington's disease: implications for pathogenesis and presymptomatic diagnosis. *Lancet* 1: 9-11, 1980. Doniger, J., Barrett, S.F., and Robbins, J.H.: A human fibroblast strain with normal survival but abnormal postreplication repair after UV irradiation. *Cancer Res.* (In press), 1980.

339. IDENTIFICATION OF HETEROZYGOUS CARRIERS OF DNA REPAIR DEFECTS

Chaganti, R. S., Sloan Kettering Inst. for Cancer Research, 410 E. 68th St., New York, New York, 10021, United States of America

OBJECTIVE: Develop, using chromosome instability (chromosome breakage and sister chromatid exchange) as the end-point, methods for the detection of the heterozygous genotypes for the genes causing the autosomal recessive disorders such as Fanconi's anemia. In addition, other syndromes such as AT, XP, may be tested as a correlate; however, the primary focus will be on determining a methodology which will detect heterozygote genotypes of FA.

340. GENETIC ANALYSIS OF HUMAN MALIGNANCY

Stanbridge, E. J., University of California, Irvine Campus, California College of Medicine, Dept. of Microbiology, Irvine, California, 92664, United States of America

OBJECTIVES: To extend our studies of the genetic control of the transformed and tumorigenic phenotypes in human cells. This includes analysis of chromosomal and extrachromosomal control of the expression of these phenotypes and determination of markers specifically associated with the tumorigenic phenotype.

BIBLIOGRAPHIC REFERENCES: Doersen, C. J. and E. J. Stanbridge. 1979. Cytoplasmic inheritance of erythromycin

resistance in human cells. *Proc. Natl. Acad. Sci. USA* 76:4549-4553.

341. GENETIC CONTROL OF NEOPLASTIC DISEASE

Gandini, E., Dallapiccola, B., Brinchi, V., Università degli Studi di Roma, Dept. of Medical Genetics, Rome, Italy

OBJECTIVES: 1) Evidence of chromosome heterogeneity in Fanconi's anemia. 2) Attempts to correct the chromosome instability phenotype of Fanconi's anemia cells by in vitro cocultivation with normal cells.

APPROACH: Chromosome instability rate in Fanconi's anemia, Estren-Dameshek type (FAED), patients has been studied in banded preparations of PHA peripheral lymphocyte cultures. Frequency and type of chromosomal aberrations, and break distribution in respect to chromosomes have been compared to those in classical Fanconi's anemia (FA). 2) Cocultivation of patients' and normal subjects' lymphocytes has been performed and the above data collected. 3) Concentrated supernatants from normal lymphocyte cultures were added to lymphocyte cultures from FAED patients and the cytogenic characterization performed as above.

PROGRESS: 1) No significant difference was found between chromosomal aberrations in FAED and FA patients (*Cancer Genet. Cytogenet.* 1980). 2) No modifications were observed in cocultivation experiments. 3) Supernatants from normal cultures did reduce significantly the instability rate in FAED patients (*Acta Med. Auxol.* 12:57-62, 1980). Experiments in progress are investigating the possible complementations between FA and FAED and among FAED supernatants of PHA peripheral lymphocyte cultures.

342. CYTOGENETICS OF CLONAL NEOPLASIAS

Hecht, F., Kaiser McCaw, B., Glover, T., Southwest Biomedical Research Inst., Cancer Cytogenetics Unit, 123 E. University Dr., Tempe, Arizona, 85281, United States of America

This research is concerned primarily with characterizing cytogenetic aspects of lymphoid malignancies and the use of specialized cells for gene mapping.

We have determined that rearrangement of the long arm of chromosome 14 is a step in the development of many lymphoid malignancies. The rearrangements involved are to be examined in more detail utilizing a series of new lymphoma cell lines, including Hodgkin's disease lines. Other chromosomal changes will be studied to further detail the nature of chromosomal changes in these diseases. In addition several lymphomas from immune deficient patients will be studied to determine whether the tumor cells contain the Epstein-Barr virus. These data will be related to the cytogenetic and cell surface findings.

The distance between the HLA loci and the centromere of chromosome 6 will be determined. This will be done using parthenogenic ovarian teratoma cells which we have determined possess only one HLA haplotype. The major chromosomal change in Burkitt's lymphoma is an 8;14 translocation. We will begin mapping genes to the long arms of chromosomes 8 and 14. These data will be used in an attempt to determine what genes are located in the segment of chromosome 8 translocated to 14.

Disease syndromes, such as ataxia-telangiectasia, are known which exhibit increased chromosome breakage. The rare genetic disease, incontinentia pigmenti, may be such a disease. Patients known to have IP will be examined to test this question.

There is some evidence that Down's syndrome children have an increased prevalence of extragonadal teratomas. This hypotheses will be tested in a large series of children with teratomas.

BIBLIOGRAPHIC REFERENCES: Wyandt, H.E., Anderson, R.S., Patil, S.R., Hecht, F.: Mechanisms of Giemsa banding. II. Giemsa components and other variables in G-banding. *Hum. Genet.* 53:211-215, 1980. Hecht, F., Kaiser-McCaw, B.: Oral clefts and childhood cancer. *Lancet* 2:145, 1980.

343. CYTOGENETIC STUDIES

Whang-Peng, J., Douglass, E., Knutsen, T., Lee, E., Bunn, P., Lewis, W., Broder, S., Magrath, I., Glatstein, E., Blaese, M., Li, F., Seiber, S., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Cytogenetic Oncology Section, 9000 Rockville Pike, Bethesda, Maryland, 20205, United States of America

The Areas of Investigation: 1. Cytogenetic studies of human neoplastic, hematological, and congenital disease. 2. In vitro cytogenetic studies of direct tumor material, tissue culture lines, and colony cultures derived or established from patients with Burkitt's lymphoma, small cell carcinoma of lung, and cancer of the brain, ovary or testis. 3. Early detection of secondary leukemia in patients with non-Hodgkin's lymphoma, CLL, small cell carcinoma of lung using chromosomal abnormalities as markers. 4. Detection of sister chromatid exchanges in families with ataxia telangiectasia, Bloom's syndrome, and a high risk of cancer.

BIBLIOGRAPHIC REFERENCES: Douglass, E.C., Magrath, I.T., Lee, E.C., and Whang-Peng, J.: Cytogenetic studies in non-African Burkitt's lymphoma. *Blood* 5: 148-155, 1980. Whang-Peng, J. and Knutsen, T.: Lymphocytic leukemias, acute and chronic. In Penington, D.C. (Ed.): *Clinics in Haematology*, 1980, Vol. 9, pp. 87-127.

344. CELLULAR AND CYTOGENETIC IMAGE PROCESSING

Johnston, D. A., University of Texas, University of Texas System Cancer Center, M.D. Anderson Hospital & Tumor Inst., Dept. of Biomathematics, 6723 Bertner Ave., c/o Texas Medical Ctr., Houston, Texas, 77030, United States of America

This is part of a broader project. A summary of this subproject is not available.

B. CYTODIAGNOSIS BY TYPE OF CANCER OR BODY SITE

1. CERVICAL AND OTHER GYNECOLOGICAL CANCERS

345. VALUE AND EFFICIENCY OF CYTOLOGY IN THE CONTROL OF CARCINOMA OF THE CERVIX

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The aim is to screen as high a percentage as possible of the 1.5 million women at risk in northwest England. To date, over 850,000 women have been examined at least once, and the project continues to determine the age of onset, frequency and interval at which cyto-testing would be best undertaken. Routine recall for screening is based on a computerized record and recall system.

346. LONG-TERM CYTOLOGICAL EVALUATION OF WOMEN USING COPPER IUDS

Engineer, A. D., Misra, J. S., Tandon, P., King Georges Medical College, Dept. of Obstetrics & Gynaecology, RH 302, Lucknow 226003, India

OBJECTIVE: The objective of a cytological evaluation of cervical smears carried out in 824 women using different types of Cu-IUDs (CuT 200, CuT 220, CuT 380, CuY, Cu 7, Multi-load and Nova T) for periods ranging from 1 to 9 years was (1) to ascertain oncogenic potential of these devices resulting from prolonged use, and (2) to determine the safest and best tolerated Cu-IUD among all the 7 types evaluated cytologically for National Family Planning Program of this country.

APPROACH: Cervical Cytology at six-month intervals was planned in 824 users after insertion of device. Endometrial aspirates were also taken prior to insertion and wherever the Cu-IUDs were removed OR changed. Routine cytological screening of patients attending Gynaec. O.P.D. of this center was also carried out to maintain a control group of comparable age (17-45 years) and parity (1-13) for matching the findings with those obtained in Cu-IUD wearers. Cervical smears from 578 women were cytologically evaluated.

PROGRESS: No case of malignancy or severe dysplasia of the cervix was encountered in any of the IUD users even after 9 years of continuous use of device in case of Cu-T 200. A total of 45 dysplastic smears were found among the smears from 824 users (5.4%) after 6 months to 8 years of use, 41 of mild and 4 of moderate degree. Regression of the lesion to normal was seen in 35 with device in situ 6-12 months later while persistence of lesion and waxing and waning patterns of dysplasia were observed in 5 and 5 cases respectively. Devices were removed in 6 of these 10 while in the remaining 4 Cu-IUDs were left in situ

due to mild nature of dysplasia, and follow-up after 6 months in 3 of these 4 showed regression of the lesion to normal in 2 and persistence of dysplasia in one.

The incidence of dysplasia was highest in CuT 380 wearers (7.9% - 8 out of 101) followed by that in multiload (7.7% - 3 out of 39) and CuY users (6.4% - 5 out of 77). The incidence of dysplasia was lowest in CuT 220 wearers (3.5% - 3 out of 88) and 5.2% in CuT 200 users (26 out of 492). The incidence of dysplasia was higher in all these 5 types of CU-IUD than the control figure of 2.8% (16 out of 578). (Text Abridged.)

347. CYTOLOGICAL FOLLOW-UP AND CLINICAL EVOLUTION IN THE EARLY STAGES OF CERVICAL DYSPLASIA

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The objectives of this project are to study the controversial aspect of the potential evolution of dysplasias as they appear spontaneously, to investigate the influence of local factors introduced by the clinician for the purpose of control or treatment, and to validate the efficacy of cervical cytology as a means of control of the process in every case, establishing for all conized tissue the correlation between the verified histologic diagnosis and preceding cytological evaluation.

All files pertaining to a private cytology laboratory that services 8 gynecologic institutions will be reviewed. A selection will be made to include all cases with a follow-up of at least 3 consecutive cytologic exams and 6 months of control, excluding cases treated with conization immediately.

In all such selected cases, a review will be made of the clinical history in order to confirm all available information and to obtain precise information on possible incidental factors in the evolution, particularly biopsies, electrocoagulation or cryosurgery. (Translated)

348. CERVICAL CYTOLOGY AND FOLIC ACID DEFICIENCY

Narvaez C., R. Davila, Noboa, Sociedad de Lucha Contra el Cancer, Departamento de Citologia, Casilla 1060, Quito, Ecuador

OBJECTIVE: To determine the possibility of cytologic diagnosis of folic acid deficiencies and use of other clinical and paraclinical findings.

APPROACH: To study cervicouterine cytomorphology in relation to folic acid deficiency.

PROGRESS: We have discovered eight cases and have performed such tests as: myelogram, in cases of obvious anemias; specific therapy, cytologic control, leukocyte polylobulation, etc.

To date we have found that specific therapy successfully reinstates normal conditions in cells having shown abnormalities due to folic acid (vitamin B conjugate) deficiency. However, dysplasias remain unchanged. No apparent relationship exists between dysplasia and cell changes caused by folic acid deficiency.

We continue accepting patients to this study in order to obtain more definitive results.

349. INVASIVE CERVICAL CANCER IN GENEVA

Raymond, L., Obradovic, M., Registre Genevois des Tumeurs, Centre de Cytologie et de Depistage du Cancer, 55 Blv. de la Cluse, 1205 Geneva, Switzerland

This investigation is a retrospective study of the frequency and chronological distribution of cytological examinations of cervical smears in women who developed invasive cervical cancer between 1970 and 1975. Cases are compared with controls, i.e., screened women who did not develop invasive cervical carcinoma, matched by age, vital data and nationality. The results of the study will help to determine the optimal interval between successive screenings.

350. CERVICAL CYTOLOGY DEMONSTRATION PROJECT FOR SOUTHWESTERN AMERICAN INDIAN POPULATION

Jordan, S. W., Key, C. R., University of New Mexico, School of Medicine, Dept. of Pathology, University Hill N.E., Albuquerque, New Mexico, 87131, United States of America

The objective of this study, in addition to providing cytological diagnostic services to a large group of American Indians

and selected non-Indians, is to investigate certain epidemiological aspects of uterine cervical and endometrial cancer. Previous work has shown significant differences in histologically established age-specific rates of cervical carcinoma between Southwestern American Indians and Caucasians, and this work will be extended and amplified. Particular interest has been directed to identification of incidence cases of cervical carcinoma and their correlation with age and gravidity or parity. Also, the natural history of cervical dysplasia is being investigated, especially the relative risk of cervical carcinoma experienced by those with the various degrees of dysplasia. Non-Indian patients attending the University of New Mexico teaching hospital and clinics serve as the control group. Records of about 170,000 patients are currently available in cytology laboratory files in machine-retrievable format.

351. RETROSPECTIVE EVALUATION OF THE PAPANICOLAOU SMEAR IN THE PREVENTION OF INVASIVE CERVICAL CANCER

Clarke, E. A., Anderson, T. W., University of Toronto, Faculty of Medicine, Dept. of Preventive Medicine & Biostatistics, 92 College St., Toronto, Ontario, M5S 1A8, Canada

OBJECTIVE: The objective of this study is to test the hypothesis, using a case-control study, that part of the decrease in the incidence of invasive carcinoma of the cervix is due to screening by the Papanicolaou ('Pap') technique.

APPROACH: Eligible cases were all patients with newly diagnosed carcinoma of the cervix admitted to a large cancer hospital during a three year period. 212 cases and 1060 neighborhood controls were interviewed to obtain information about risk factors and all 'Pap' smears taken in the previous ten years. Subsequently, confirmation of the 'Pap' smear history was obtained from attending physicians. Significantly fewer of the cases (27%) than the controls (52%) had had at least one 'Pap' smear in the absence of symptoms in the five years prior to diagnosis of the index case (p less than 0.0001). Expressed as a risk ratio, these figures indicate that asymptomatic women who have not had any 'Pap' tests in the previous 5 years are at 3.3 times the risk of developing symptomatic cervical cancer. Controlling for known risk factors such as socio-economic status, age at first intercourse, etc., the risk remains constant.

352. MECHANISMS AND MANIFESTATIONS OF CARCINOMATOUS CHANGE IN HUMAN AND EXPERIMENTAL CANCER

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The research reported covers a multidisciplinary program for the early diagnosis of human cancer and for studying metastatic properties of cellular attachment. The attachment of epithelial cells and of fibroblasts to surfaces was studied by a newly modified reflexion contrast microscope. The malignant potential of cervical condylomata accuminata was assessed in a patient population of 190,000 well women. A third study concerns the identification of primary human breast cancers with their metastases using quantitative measurements of individual cellular DNA distribution. A fourth study has been involved in establishing new morphometric and cytochemical markers for highly differentiated urothelial cancers. The potential of fine needle aspiration biopsy for obtaining new parameters for estimating prognosis and further diagnosis of primary and secondary human cancers was explored. A continuing study of carcinogen metabolism included effects of anti-oxidants on metabolism of polycyclic aromatic hydrocarbons. A study of the kinetics of cellular growth in cervical epithelium was conducted to determine the viability of cervical epithelium after exposure to carcinogens.

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353. CONDYLOMA ACUMINATUM AND CERVICAL INTRAEPITHELIAL NEOPLASIA

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Recent studies suggest that the papova virus is related to the genesis of cervical intraepithelial neoplasia (CIN). A long term prospective study is planned to verify this hypothesis. Patients with a clinical diagnosis of condyloma acuminatum will be examined using cervical cytology and colposcopy. All atypical areas will be biopsied. Examination will take place at the moment of diagnosis and thereafter every six months for five years. An estimated number of 100 patients will be included each year for five years. A control group will be formed by patients of the same age and socio-economic status. Data obtained will also be compared with the incidence of CIN in the general population as shown by screening.

354. COLPOSCOPIC SCREENING IN A UNIVERSITY COMMUNITY (CERVICOUTERINE)

Aruta, C. J., Universidad de Chile, Area Ginecologica, 1058 Av. Bernardo O'Higgins, Santiago, Chile

OBJECTIVE: To develop a method for detection of cervicouterine cancer that is both efficient and economical for our university community.

METHOD: Since 1976 we have been developing a routine colposcopic screening program offered free of charge to users. The results are not the same with the Papanicolaou, which should be paid for by patients, most of whom have modest economic resources. In any case the cytologic method is reserved for those cases of doubtful colposcopy or for those who present with squamo-cylindrical intracervical junction, which can not be seen by endospeculum.

Our experience over five years is showing the yield of this type of consultation to be the most satisfactory. (Translated)

355. MORPHOMETRIC MEASUREMENTS IN HUMAN CANCER

Wiernik, G., Bradbury, S., Spriggs, A. I., Cowdell, R. H., Plant, M., University of Oxford, Research Inst., c/o Churchill Hospital, Oxford OX3 7LJ, England, United Kingdom

OBJECTIVES: Quantitative microscopy of carcinoma of the human cervix uteri and normal cervical tissues in order to achieve an earlier correct prediction of the development of an invasive tumor.

APPROACH: Morphometric analysis has been performed of squamous epithelial cells in biopsy specimens obtained either from patients with proven invasive squamous cell carcinoma or from patients with histologically proven normal cervixes. This has shown that when this data is submitted to a programme of multivariate analysis a highly significant difference can be demonstrated (Wiernik, Bradbury, Plant, Cowdell and Williams, 1973, Brit. J. Cancer, 28, 488-499). Parameters that have been measured include nuclear area, ribosomes, fibrils, surface density, inter-cellular space, etc. The methodology is currently being adapted for the direct examination of cervical smear specimens.

PROGRESS: In order to obtain data concerning the pre-malignant phases of cervix uteri disease, cone biopsy specimens from patients with dysplasia and carcinoma-in-situ are being analysed and will be compared with the data from the normal and invasive cancer specimens. Cervical smears taken at the time of diagnosis of patients with normal histological features and of patients in whom a histological diagnosis of invasive squamous cell carcinoma was made are being analysed by a modified methodology to establish a similar quantitative discrimination. Cervical smears from patients now presenting with invasive carcinoma, taken over the previous decade or more, are also being analysed in a similar manner to ascertain the earliest time when reliable predictive data can be derived.

356. POSSIBLE ROLE OF VITAMIN A IN UTERINE CERVICAL DYSPLASIA

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In this study of carcinoma of the uterine cervix the primary focus is on the possible interrelationships of diet, with emphasis upon vitamin A, social environmental epidemiological

factors, and the cellular changes which are detectable clinically by routine cytology and biopsy of the uterine cervix. The availability of the Papanicolaou test permits screening of large population groups and the finding of a positive or suspicious smear identifies 'women at risk'. Such women are being studied intensively in terms of (1) diet, with computer coding and scoring of nutrient values, (2) ethnic and socio-economic background and (3) personal lifestyle including educational performance as well as (4) measurements of specific blood and tissue retinol and retinoic binding proteins.

357. NATURAL HISTORY OF DYSPLASIA OF THE UTERINE CERVIX

Miller, A. B., Thompson, D. W., Jain, M., University of Toronto, Dept. of Preventive Medicine & Biostatistics, Medical Sciences Bldg., Toronto, Ontario, M5S 1A8, Canada

In most models of the natural history of squamous carcinoma of the cervix, dysplasia is regarded as the first recognizable pathological process in an orderly progression from normal to malignant epithelium. Since dysplasia does not always become malignant, there is need to clarify the extent to which regression forms part of the natural history of dysplasia. The present study aims at seeking information on the natural history of cytologically identified but untreated dysplasia in comparison with a sample of normal women with smears examined in the same laboratory. An estimated 10,000 women in the case series of moderate or severe dysplasia and 30,000 in the control series will be included. Information is also being sought on the possible association of oral contraceptive use and the risk of progression of dysplasia. Data extraction is currently under progress.

358. EPIDEMIOLOGY OF PRECANCEROUS AND EARLY NEOPLASTIC LESIONS OF THE UTERINE CERVIX AND CORPUS

Munteanu, S., Institutul Oncologic Cluj-Napoca, Dept. of Early Diagnosis of Uterine Cancer, 34-36 Republicii, 3400 Cluj-Napoca, Romania

The objective is to examine the relationship between age at first sexual intercourse, age at first birth, marital status, number of births before age 19 and other known aetiological and epidemiological factors and cervical and corpus neoplasia. A form containing 38 groups of questions, all coded, is filled in by any patient attending the Colposcopy Early Diagnosis Clinic, an out-patient clinic in the Oncological Institute. The following investigative methods are used: Physical examination, various cytological and biopsy examinations, colposcopy, and a thorough inquiry into sexual activity. These are repeated periodically. About 2,500 patients with various benign and malignant cervical lesions and 300 patients with endometrial carcinoma have been recorded and are involved in a long-term follow-up study.

359. CORRELATION BETWEEN CYTOLOGIC AND HISTOPATHOLOGIC STUDIES IN DYSPLASTIC AND NEOPLASTIC LESIONS OF THE UTERINE CERVIX

de Borges, R. J., Hospital Vargas, Departamento de Citologia, San Jose, Caracas 101, Venezuela

OBJECTIVE: The objective of this project has been to determine the degree of correlation between the cytologic evaluation and histopathologic diagnosis in the Hospital Universitario General of the city of Caracas. Since the establishment of the degree of correlation, cytodiagnosis has been used to determine the incidence of these lesions in the female population attending the various departments of the hospital.

APPROACH: As described in the previous paragraph, cytology and histopathology have been used to establish the statistical correlation between the two diagnostic methods. After determining the significance of the different cytologic changes, we have used this method to determine the incidence of dysplastic and neoplastic lesions of the uterine cervix.

PROGRESS: To date, we have studied 80,000 gynecologic cytological specimens. In 400 cases with alterations indicative of tumor (200) or dysplasia (200), a comparison was made with histopathologic diagnosis of these cases. Confirmation was obtained for 70% of the dysplasias and 95% of the lesions diagnosed as malignant in the first biopsy. Of the 80,000 cases studied, incidences of 2.8% dysplasias and of 1.4% malignant lesions were found with cytodiagnosis. We intend to continue adding cases to this study. (Translated)

360. EVALUATION OF EARLY DETECTION OF CANCER OF THE CERVIX UTERI BY CYTOLOGICAL SCREENING

Neuser, D., Städtisches Krankenhaus im Friedrichshagen, Zytodiagnostisches Zentrallabor, Leninalle 49, 1017 Berlin, German Democratic Republic

Between 1955 and 1969 the situation with respect to cervix carcinoma in Berlin hardly changed; morbidity, mortality, stage distribution and 5-year survival rates remained much the same. It is proposed to test whether cytological screening can lower morbidity and mortality. For this purpose, all women aged 20 to 65 living in Berlin will be cytologically examined every two years. Using the National Central Cancer Registry, the relationship of carcinoma in situ to invasive carcinoma and the stage distribution of the invasive cervix carcinomas and morbidity and mortality before, during, and after screening will be investigated. The first four years were characterized by a considerable increase in carcinomas in situ and a shifting of stages in favor of stages Ia and Ib, with coverage of 80% of the target population. In 1978 a significant decrease in carcinoma in situ (38%) and in invasive cervical cancer (30%) was observed for the first time in the whole female population.

361. BIOPEPR - SCREENING OF CERVICAL SMEARS

Van de Walle, R. T., Vooy, G. P., Zahniser, D., Oud, P., Raaymakers, M., Katholieke Universiteit Nijmegen, Physics Lab., High Energy Nuclear Physics Section, Toernooiveld, 6525 ED Nijmegen, Netherlands

OBJECTIVE: To develop a fast (approximately 100 smears/hr) apparatus for pre-screening of cervical smears with a false alarm rate of 10 percent or less and a missed positive rate of 1 percent or less.

APPROACH: A cathode-ray tube scanner looking at (enlarged) pictures of smear-fields, derived from an instrument developed in high energy physics for the analyses of bubble chamber film; development of special preparation and staining techniques in order to obtain smears suitable for fully automatic scanning.

PROGRESS: In a sample of approximately 200 smears measured at speeds between 120-180 smears/hr and with a missed positive rate of less than 1 percent approximately, a false alarm rate of approximately 18 percent was obtained (results reported at the 6th Int. Conference on Automated Cytology - Elmau, April 1978). Field test on a sample from more than 5,000 smears is now in progress.

362. AUTOMATION OF PRESCHOOLING PROCEDURES FOR CERVICAL CARCINOMA AND HEMATOLOGICAL DISORDERS WITH TV ANALYSIS

Ploem, J. S., Al, I., vanIngen, E. M., Leyte, L., Ploem, J. J., Vrolijk, J., Tanke, H. J., vanDriel, A. J., Koper, C. J., Verwoerd, N. P., Wielenga, G., Rijksuniversiteit te Leiden, Sylvius Labs., Dept. of Histochemistry & Cytochemistry, Wassenaarseweg 72, P.O. Box 9503, 2300 RA Leyden, Netherlands

The objective of the research is a rapid automated screening procedure for cervical carcinoma and premalignant lesions of the cervix. At first, a semi-automated investigation was performed on a large clinical material consisting of 772 cervical smears. These smears were visually screened for cells with a visually expected high DNA content. The fluorescence of these cell nuclei was subsequently measured. It appeared that about 10% of the negative smears contained cells with a DNA content of more than 5C (false positives). In the positive material (moderate/severe dysplasia and more serious lesions), 95% of the smears contained cells with such a high DNA content (5% false negatives). This criterion thus seemed suitable for use as one of the preschooling criteria. We found an increased contrast of nuclear chromatin structure a second useful criterion in a preschooling program for cervical cancer. It was possible to implement artifact rejection procedures in our television analysis computer (LEYTAS) which resulted in an efficient elimination of artifacts. The mean number of non-rejected artifacts on plus or minus 20,000 analyzed objects was only 5. A total of 86 negatives and 139 positives were analyzed with LEYTAS. The whole screening procedure is carried out without intervention of the operator, meaning that autofocus, transportation of the stage, detection of objects, rejection of artifacts, and accumulation of relevant data such as the location of detected non-

rejected objects are fully automated. At the end of the procedure the detected non-rejected objects can be visualized in the preparation as well as on TV screen. By visual judgement the artifacts are then separated from the single cells, which can be further divided into negative, suspicious and positive. Further implementation of criteria concerning chromatin structure is at present being developed. The result of the 225 preparations that were screened with LEYTAS on increased DNA content and chromatin contrast resulted in a false negative ratio of about 2%.

363. FLOW-CYTOMETRIC PRESCHOOLING FOR CERVICAL CANCER DETECTION

Linden, W. A., Baisch, H., Scholz, K., Universität Hamburg, Institut für Biophysik und Strahlenbiologie, Martinistrasse 52, 2000 Hamburg 20, Federal Republic of Germany

OBJECTIVE: To develop an automatic cytophotometric preschooling method for detection of cervical cancer.

APPROACH: Cell suspensions from cervical smears are analyzed by flow cytometry (FCM). The cellular DNA is stained with 4'-6-diamidino-2-phenylindol (DAPI) and the protein with sulfur-rhodamine (SR 101). The analysis is based upon the increased DNA content of malignant cells and the different nuclear/cytoplasmic ratio. Each two-parameter FCM measurement is evaluated mathematically to discriminate between normal and suspicious samples.

PROGRESS: Our previous investigations have shown that measurement of DNA content alone is not sufficient (Linden W.A., Ochlich K., Baisch H., Scholz K.-U., Mauss H.-J., Stegner H.-E., Joshi D.S., Wu C.T., Koprowska I., Nicolini C., Flow cytometric preschooling of cervical smears. *J. Histochem. Cytochem.* 27, 529-535 (1979)). Comparing the FCM results with the cytologic diagnoses after Papanicolaou staining we obtained 26% false positives admitting 11% false negatives. In the present study a field test with two-parameter FCM measurements is being conducted. Criteria for mathematical analysis of histograms are evaluated to identify suspicious subpopulations.

364. CASE-CONTROL MONITORING OF CERVICAL SCREENING PROGRAMS IN NORTHERN ITALY

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Large scale cytological screening programs were developed at the end of the sixties. Due to the very long natural history of cervical neoplasia a significant effect on cervical cancer mortality is not yet expected; an effect on the incidence of invasive cancer however might be already observable. If the programs were able to detect and to provide adequate treatment of preinvasive lesions, theoretically one would expect to find less cytological examinations in the history of invasive cervical cancer cases than among healthy women. A case-control investigation is being conducted in which all new cases of invasive cervical cancer occurring in the Milan population and three hospital controls per case are linked with the files of cytological services in order to identify the women which have been previously submitted to cytological examinations. Age and social class will be considered as cofounders and a correction will be introduced to take into account the fact that, as the invasive cervical cancer may have been present for a long time before the diagnosis is made, incident cases are selected as those who have not been previously submitted to screening smears, as these would have been diagnosed as cases before the beginning of the study period.

365. THE YORKSHIRE CERVICAL CYTOLOGY SCREENING PROJECT

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Data have been collected on a random 10% sample of all cervical smear examinations carried out in two areas of West Yorkshire in the years 1976-1977 (9,725 cases). Information includes demographic and clinical details and cytological results. In addition a separate 40% sample of all cytologically positive smears (1,139 cases) has been used to provide more detailed data on pathological diagnosis and patterns and results of follow-up tests. This survey material yields estimates of the screening

activity in the study areas, and quantitative results of testing. It is possible to estimate incidence rates of pre-clinical abnormalities (dysplasia, carcinoma in situ) as well as point prevalence. The data are being used in a computer simulation model of the screening process. Currently this has been developed for analytical and educational purposes, by exploring the logical consequences of hypotheses about the disease and the effects of intervention. The incorporation of data from the cytology surveys will allow the use of the simulation model in a predictive mode as a tool in local and national planning.

366. OPTIMIZATION OF SCREENING FOR CERVIX CANCER

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Cancer of the uterine cervix contributes significantly to the total cancer morbidity and mortality among women in the United States. Cytologic screening using the Papanicolaou smear has become a widely accepted modality for detecting cervical neoplastic disease. The cost-effectiveness of Papanicolaou smear screening on an annual basis has been questioned. Recently, recommendations have been made for modifying current practices by increasing the interval between successive cytologic examinations among those women identified as being at 'low risk' of incidence. In no instance have the sequelae of such proposed changes been examined in terms of expected changes in incidence, survival and mortality for cancer of the cervix.

The natural history of cervical neoplastic disease has been described by us and other investigators. Several cytologically defined lesions are thought to be precursors of invasive cancer of the cervix and there is wide acceptance that carcinoma-in-situ is antecedent to invasive disease. This lesion is potentially 100% curable and will, if untreated, progress to invasive cancer in the majority of cases. The transition times and probabilities of the stages of cervical neoplastic disease have been presented previously. Using these data together with age-specific incidence rates currently available from a variety of sources and available estimates of incidence, the studies proposed here will generate estimates of the incidence, survival and mortality for cervix cancer among defined populations exposed to specified cytologic screening regimens.

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367. CARDIFF CERVICAL CYTOLOGY STUDY

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The main objective of the Cardiff Cervical Cytology Study is to evaluate screening for cervical cancer. The study is based on a defined population: all women who have ever been married, aged 25-69, resident within the Cardiff City area. The basic design objective was that all eligible women should be screened three times. The defined population was enumerated by house-to-house visiting, and eligible women were offered appointments for a cervical smear test at specially organized clinics. The study began in late 1965 and the first round of visiting the city finished early in 1971. The defined population numbers some 71,000 women of whom 46,000 (65%) accepted the offer of the test. Subsequent to the first smear women were re-called for second and third smears at two to three yearly intervals. Some 38,000 have so far had a second smear and over 30,000 have had a third. The study should provide estimates of the prevalence and incidence of the various pathological states, and estimates of false negative and false positive error rates. Death and registration rates for invasive cancer in the Cardiff City area are being monitored.

368. NATURAL HISTORY OF CERVICAL CANCER

Green, G. H., Noda, S., Wright, L. H., University of Auckland, Postgraduate School of Obstetrics & Gynecology, P.O. Box 2175, Claude Rd., Auckland 3, New Zealand

The aim is to ascertain whether or not cervical cancer commences in the pre-invasive form and whether or not the

discovery of carcinoma in situ by cytology will decrease the mortality rate of invasive cervical cancer in the population. The study population consists of all cases of in situ cancer diagnosed and treated at National Womens Hospital, Auckland, New Zealand, (about 150 per annum); all cases of invasive cancer (about 50 to 60 per annum) occurring in populations served by Auckland Hospital Board (approximately 890,000 in 1978), and all cases of in situ and invasive cancer and their district of origin notified to the National Cancer Registry, Department of Health, Wellington. All clinical and sociological data relative to cases occurring in the Auckland Hospital Board area will be correlated and age-standardized incidence and mortality rates, and trends in these will be calculated for all New Zealand cases. A cytology screening index (No. of smears per 1,000 population aged 20 years and over) will be used in association with these analyses. In addition to Auckland figures (approximately 150 in situ and 60 invasive cancers per annum), the New Zealand totals (300 in situ, 150 invasive cancers approximately per annum) are being collected for analysis. A recent publication (December 1978) reveals that the incidence and mortality rates for young New Zealand women (20-34 years), the most heavily cytologically screened group in the country, have been rising since about 1960. The stage distribution in all age groups has not altered since cytology screening was introduced into New Zealand in 1955.

369. CERVICAL CYTOLOGY PROGRAM

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Arkansas ranks 12th among the states of the U.S.A. with regard to mortality from uterine cervical cancer. The existence of rural communities, non-white populations and areas with low physician:population ratio constitutes high risk conditions. There is insufficient screening and follow-up. The program is designed to reach the high-risk woman by utilizing ancillary facilities in statewide local health departments, public health nurses and a system of newly developed colposcopy clinics. These clinics provide for definitive diagnosis for about 85-90% of women found to have abnormal Papanicolaou smears. The program aims to follow up (1) all women with abnormalities to ascertain the course of dysplasias of the cervix and (2) all women with normal cytological smears prospectively in relation to epidemiological factors, socioeconomic status, oral contraceptives and other factors.

370. AUTOMATION OF DIAGNOSTIC CANCER CYTOLOGY FOR SCREENING OF UTERINE CANCER USING IMAGE ANALYSIS SYSTEM - DEVELOPMENT OF AUTOMATED SYSTEM 'CYBEST'

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OBJECTIVE: Since 1967 we have been studying cytology automation for screening of uterine cancer assuming the following: 1) Image analysis system enables examination of specimens processed by machine directly under the microscope. 2) Cell dispersion pretreatment of samples, smearing, staining, etc. should be also automated simultaneously. 3) False negative rate is within that of human screeners and false positive - 20% or 30%. 4) Time required is within 3 minutes per specimen. 5) System should mainly use a flexible software program, etc.

APPROACH: In 1972 a CYBEST (Cytobiological Electronic Screening System) prototype was developed following fundamental studies of feature extraction, feature evaluation using ambiguity differential function, and segmentation of cell and nuclear images. Model 2 was developed in 1974 with an improved mechanism and function. The parameters employed are nuclear size, nuclear optical density, N/C ratio and nuclear shape. Model 3 was developed in 1978 after repeated field tests and incorporated the improved program.

PROGRESS: Model 2 employs a flying spot scanning device with a coarse scan 4 micrometer and a fine scan 1 micrometer spot. After successful automatic shading, correct control and the quick removal of after-image from TV, the TV scanning system, Model 3 was developed. This system has rapid processing mechanisms such as quick autofocussing device, rapid slide transfer to the scanning stage, etc., which contribute

to the rapid processing (3 minutes per specimen). Model 3 can automatically process 200 samples in 10 hours. Extensive field tests are carried out at this time.

371. CLINICOPATHOLOGICAL AND HISTOCHEMICAL STUDY OF OVARIAN CLEAR CELL CARCINOMA

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OBJECTIVE: Ovarian clear cell carcinoma is the tumor with bad prognosis. Although the histogenesis of this type of tumor has been discussed it is still being unclear. Little is known about the histochemistry of mucins secreted by these carcinomas.

APPROACH: The contents of the mucins are differentiated by histochemical analysis. All the cases found in the Tampere district between 1964 and 1978 are carefully analyzed and case records are examined. Correlations between the tumors, their histochemistry and the course of the disease, as well as the clinicopathological relations, are discussed.

PROGRESS: The work has been completed and the results will be published in International Journal of Gynaecology and Obstetrics in the autumn of 1980.

372. IMMUNODIAGNOSIS FOR EARLY DETECTION OF OVARIAN CANCER

Stolbach, L. L., Tufts University, Boston Campus, School of Medicine, Dept. of Medicine, 136 Harrison Ave., Boston, Massachusetts, 02111, United States of America

OBJECTIVE: Establish an immunodiagnostic method of identifying the existence of ovarian cancer at an early stage of the disease and for serial monitoring of patients with ovarian cancer so that treatment can be more effective. Specifically, the Contractor will have available an immunologically oriented laboratory capable of recreating, identifying, and purifying antigens and creating antisera; he will have radioimmunoassay capability in order to perform large numbers of tests on patients; he will have access to patients with carcinoma and will locate, identify, and have access to a population cohort at high risk for ovarian cancer, including expected laparotomy for suspicion of ovarian cancer.

373. TRENDS IN MORBIDITY AND MORTALITY OF UTERINE CANCER IN RELATION TO MASS CYTOLOGICAL SCREENING

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A population-based registry of all uterine tumors in Jefferson County, Kentucky, has been maintained since 1953. Jefferson County has had mass cytological screening since 1956 and by as early as 1967 over 90% of the population had been screened. Morbidity and mortality trends as well as patient survival and a record of histological type have been monitored. Over the period of the study mortality from cervix cancer has decreased significantly when compared to mortality trends in United Kingdom and Ireland and in Denmark. The study continues. Several papers on this topic are in preparation or have been published.

374. DIAGNOSTIC HISTOCHEMICAL AND ELECTRON MICROSCOPIC FEATURES OF HUMAN OVARIAN TUMORS

Klemi, P. J., Turun Yliopisto, Dept. of Pathology, Kiinamyllynk 8-10, SF20500 Turku 52, Finland

OBJECTIVE: Improvement in diagnosis of ovarian tumors. In order to improve the diagnosing of ovarian tumors various histochemical and electron-microscopic methods will be used.

375. IMMUNOHISTOLOGIC STUDY OF UTERINE CANCER

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Extensive studies of breast cancer, including immunoperoxidase studies in our own laboratory, have attempted to correlate expression of hormone receptors and tumor-associated antigens with selection of therapy and eventual clinical outcome. By

comparison, studies of cervical and endometrial cancer are few and far between.

The majority of correlative marker studies of gynecological neoplasms have measured either serum factors or tissue homogenate antigens, thereby precluding direct correlation between cell staining patterns and standard morphologic criteria used in the diagnosis of malignancy. Immunoperoxidase techniques applied to fixed embedded tissues offer the prospect of direct visualization of antigens in standard histologic sections, and so offer the diagnostic pathologist the opportunity of simultaneously identifying normal and neoplastic cells both by their morphologic characteristics and by their antigenic constitution.

The aim of the proposed study is twofold. First, we plan to extend the current preliminary immunoperoxidase study of steroid hormone receptors in gynecologic neoplasms to 50 cases each of endometrial and cervical carcinoma. The pattern and intensity of tumor cell staining for estrogen receptor, as determined by immunohistochemical methods in a retrospective study will be compared with histologic findings and the clinical outcome of the disease. In the second phase of the proposed program, the study will be broadened to include examination of the same case population for other possible tumor markers (e.g., carcinoembryonic antigen (CEA), human chorionic gonadotropin (HCG), pregnancy-specific beta₂ glycoprotein, lactoferrin, alpha fetoprotein, T-antigen); the potential diagnostic and prognostic significance of the interrelationships between these potential markers and the hormone receptors will be investigated.

We anticipate that the combined immunohistochemical approach will lead to more precise diagnosis, to more effective discrimination between benign hyperplastic conditions and neoplasia, and to more effective selection of therapy.

376. THE NUCLEAR DIAMETER IN THE DIAGNOSIS OF ENDOMETRIAL ADENOCARCINOMA

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Longitudinal and transversal diameters were measured in nuclei of 1,253 normal endometrial specimens and 1,213 endometrial adenocarcinoma specimens naturally exfoliated and collected in routine cervical smears. The determinations were performed blindly by two investigators who obtained the following mean diameters, respectively: longitudinal, 8.28 and 8.40 microns and diagonal, 6.44 and 6.64 microns for normal cells; longitudinal, 11.26 and 11.61 microns and diagonal, 8.97 and 8.87 microns for neoplastic cells.

Significant statistical differences in both populations were obtained, comparing the two diameters as independent variables. The feasibility of applying this simple method as a parameter in the cytological diagnosis of endometrial adenocarcinoma is currently under study.

377. ASSESSMENT OF TECHNIQUES FOR ENDOMETRIAL CANCER DETECTION

Bibbo, M., Herbst, A. L., Kluskens, L. F., University of Chicago, Pritzker School of Medicine, Dept. of Obstetrics & Gynecology, 5801 S. Ellis Ave., Chicago, Illinois, 60637, United States of America

Three techniques, namely, the endometrial aspiration (Vacutage), the endocervical aspiration and the routine vaginal, ectocervical and endocervical cytologic smears, are being used simultaneously to detect endometrial cancer and its precursors in an academic institution and in a community hospital and offices of gynecologists. We propose to enlarge the sample for the ongoing detection phase of the study at the University of Chicago; to study the optimal fixation technique for specimens which have to be transported to the University of Chicago for processing and to test the performance level of the three techniques in a community setting. This will involve a preparatory stage and a main stage.

378. CYTOLOGICAL MONITORING OF WOMEN WHO USE COPPER-CONTAINING INTRAUTERINE DEVICES

Luthra, U. K., Devi, P. K., Engineer, A. D., Bhinder, G., Philips, F. S., Virkar, K. D., Indian Council of Medical Research, Cytology Research Centre, c/o Maulana Azad Medical Coll., New Delhi 2, India

There are controversial reports on the potential carcinogenic effect of various intrauterine devices on female genital tract epithelia. In India copper-containing IUDs are currently widely used for contraception. In order to rule out any carcinogenic effect on the female genital tract, this study was conducted.

Cytological monitoring of 2,603 women who use various types of copper-containing IUDs was conducted along with clinical trials followed up to 60 months of use. From the data, it was concluded that: (i) There were no significant alterations in the female genital tract epithelia due to usage of Cu-IUD for 60 months. (ii) It is not mandatory to treat the women who show cytological evidence of inflammation before the insertion of Cu-IUD, as the risk for development or progression of dysplastic lesions in these women did not differ significantly from the risk for those with negative smears on usage of Cu-IUD for 60 months.

379. DEVELOPMENT OF AUTOMATED CELL RECOGNITION SYSTEM

Wied, G. L., University of Chicago, Pritzker School of Medicine, Dept. of Pathology, 5801 S. Ellis Ave., Chicago, Illinois, 60637, United States of America

OBJECTIVES: Feasibility study on optimizing selection of wavelengths for three-color scanning of Papanicolaou-stained gynecologic specimens. Supply NCI with industry-compatible magnetic tapes containing digitized raw image and edited images of all cells studied under this contract accompanied by identifying and diagnostic information similarly recorded on an ongoing basis and by photomicrographs. Replacement of the current green filter in the new three-color scanning system by an interference filter id. Brief study using the three-color system in which only unequivocal gynecologic cytopathology specimens are used as the 'learning' data base.

380. CECAR - A CYTOHISTOLOGICAL METHOD (ABBREVI)

Bocanera, R. A., Schlaen, I., Roncoroni, E., Canaves, L., Villa, H., Freyre Hospital, Olive 1151, Mendoza 1670, 2000 Rosario, Argentina

The CECAR (for 'cepillo,' brush; 'endometrial,' endometrial; 'cabezal,' bolster; 'articulado,' articulated; 'rotatorio,' rotating) method was applied to 427 women whose ages ranged from 20 to 91, with the average age being 48.2 years. The material thus obtained allowed cytological and histological evaluation by means of conventional cytohistological procedures. Useful material was obtained for diagnosis in 98.4% of the cases. In 89 patients out of the total number of women examined D and C was performed after the CECAR application. False negatives were not found. In five patients, after the cytological evaluation obtained with CECAR, adenocarcinoma of the endometrium was suspected. However, it was not confirmed by histological evaluation obtained with the same CECAR, nor by the D and C, both showing the presence of endometrial polyps.

RESULTS: Twenty-eight benign hyperplasias (simple hyperplasia and cystic glandular hyperplasia), 11 early endometrial lesions, (adenomatous hyperplasia, atypical hyperplasia and adenocarcinoma in situ), and 13 infiltrative endometrial adenocarcinomas were found. The average age for women with carcinomas was 64.8 years, and for women with early endometrial lesions - 49.5 years. Out of 24 patients with early endometrial lesions and carcinomas, 4 did not demonstrate any symptoms at all.

381. PROGRAM OF PREVENTIVE TOCOGYNECOLOGY - PILOT DESIGN FOR INTEGRAL MEDICAL CARE

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OBJECTIVE: Three years ago a program was initiated for performing a gynecologic checkup of all women visiting the Hospital das Clinicas of the Universidade Estadual de Campinas for any reason whatsoever. The objective of the program is to detect asymptomatic gynecologic pathology and study the viability of a method of choice that would, to a certain point, have a place in a network of primary health care delivery.

METHOD: Using a very simple methodology, 7,320 women were examined by medical students and obstetrical nurses under a physician's supervision. Some form of gynecologic

pathology or a condition of high risk was detected in 22.41% of the patients: 0.31% had breast cancer; 0.72% had positive cytologic exams; 11.7% had uterine myoma; and 4.04% had severe genital dystopia.

The routine gynecological checkup has been shown to be useful and important for all women visiting the hospital for any reason; the organizational and economical requirements of this type of service are justifiable.

A similar automatic checkup of male patients is recommended, in order to detect diseases of greatest prevalence. (Translated)

2. LEUKEMIA AND LYMPHOMA

382. MORPHOMETRIC ANALYSIS OF BONE MARROW BIOPSIES IN ACUTE LEUKEMIA

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OBJECTIVE: To obtain quantitative data about bone-marrow cellularity in leukemia patients, morphometric measurements at bone-marrow biopsies are performed before, during and after cytostatic therapy.

APPROACH: Biopsies, taken from the posterior iliac crest, are embedded in methyl-metacrylate. Sections are stained and microphotographed. The diapositives are projected on a suitable grid and the number of test points on the different cell types are counted as well as the number of test line intersections. From these data the cellularity as well as the ratios of the different cell types can be calculated.

PROGRESS: Biopsies from ten acute leukemia patients were embedded before, during and after therapy. All technical problems of morphometric analysis have been solved. Statistical analysis revealed the number of sections and the number of fields in each section that must be analyzed. At the end of 1978 morphometric analysis of patients' material started.

383. DIAGNOSTIC AND PROGNOSTIC FACTORS IN PRELEUKEMIC STATES

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OBJECTIVE: To find the common kinetic factors for diagnosis of preleukemic states in anemias and leucopenias and the data indicating a leukemic transformation in these cases and chronic myeloid leukemia.

APPROACH: Sequential studies of bone marrow culture in a semisolid medium, autoradiography after in vivo labelling with ³H-thymidine, evaluation of bone marrow cellularity with ⁵⁹Fe - kinetics and bone marrow scintigraphy.

PROGRESS: Transformation in acute leukemia has been seen to be indicated by a decrease in total bone marrow cellularity and, in immature granulocytes, Labeling Index for some months to years and by a decrease in colony formation in cultures with an increase in cluster/colony ratio for a few weeks. Therefore, each of these studies is useful for the short- and long-term prognosis and for therapeutical decisions. However, results seem to be different in various aspects of preleukemic cytopenias. Further studies are being developed.

384. PATHOLOGY OF CELL DIFFERENTIATION IN LEUKEMIA

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Our overall goal is to gain new information on the abnormal differentiation and function of leukemic cells and to establish better methods for the diagnosis and classification of acute leukemias. Major techniques to be applied are light-(LM) and electron-microscopy (EM) and cytochemistry, scanning EM, and tissue culture. We have established a firm theoretical and technical basis for the proposed research by our previous work on the differentiation and functions of normal leukocytes. Specifically, the objectives are: 1) To ascertain if defects in acute leukemia cell maturation correlate with 'blocks in differentiation' at stages of secretory pathway by analyzing the localization of peroxidase and other granule enzymes; 2) using LM, EM and histochemistry, to subclassify acute leukemias into distinct

cell lines, with emphasis on developing and testing new methodologies.

385. CYTOPATHOLOGICAL STUDY ON MALIGNANT LYMPHOMA USING MICROPHOTOMETRY AND CELL CULTURE

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OBJECTIVE: To delineate the pathological and cytopathological characteristics of various kinds of malignant lymphomas as well as to differentiate and evaluate their malignancy, cytomorphological and cytobiological features were extracted and the results were correlated with the histological classification of lymphoma. Follicular lymphomas are often difficult to differentiate from reactive hyperplasia and differentiation between the benign and malignant forms is also difficult. Reticulum cell sarcoma is frequently difficult to differentiate from lymphosarcoma. To find a solution to these problems, research and experimental studies are carried out as described below.

APPROACH: In addition to the routine histology sections, imprint smears were made from the cut surface of the biopsied and/or autopsied lymph nodes or lymphoid tissue. They were stained with Feulgen reaction; besides various routine stainings Olympus MMSP (microphotometer) was provided to measure the nuclear DNA content of the Feulgen-stained materials. Electron-microscopical observation and the method for T- and B-cell marker detection were applied to various kinds of lymphomas.

PROGRESS: Comparing the histograms of the nuclear DNA content of various kinds of lymphomas, characteristic features were disclosed as follows; mature lymphosarcoma and follicular lymphomas show a pattern close to the normal range, but premature and pleomorphic cell types show wide distribution in ploidy on the histogram. Hodgkin's cells and/or Reed-Sternberg giant cells often show a pattern resembling that of malignant mode. Data on various lymphomas are being accumulated.

386. CYTOBIOLOGICAL INVESTIGATIONS ON LYMPHOMA CELLS - DIAGNOSTIC AND PROGNOSTIC SIGNIFICANCE

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The purpose of the present investigation is to evaluate whether the study of lymphomatous cell populations carried out in parallel with cytokinetic, cytoimmunological and cytochemical techniques may provide additional information compared with that obtainable from the application of the single methods of study mentioned. To this end kinetic activity has been measured employing radioactive nucleic acid precursors, the mitotic index and the DNA cellular content determined by microdensitometry; the cytoimmunological features have been evaluated by the use of rosetting techniques, specific antisera and immunohistological methods, while the cytochemical pattern of malignant cells has been studied employing the methods currently in use for recognizing the different types of lymphomatous cells (PAS, acid and alkaline phosphatases, ANAE, alpha-naphthyl butyrate esterase, etc.).

Preliminary results obtained in 23 cases of non-Hodgkin's B lymphomas have already been published (*Acta Haematologica*, 64:148-153, 1980) and appears to indicate the possibility of obtaining diagnostic and prognostic information on the basis of the combined method of study employed.

387. PROGNOSTIC FACTORS IN NON-HODGKIN'S MALIGNANT LYMPHOMAS WITH EMPHASIS ON THE FUNCTIONAL APPROACH IN CLASSIFICATION OF LYMPHOMAS

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The summary for this project is under entry no. 305.

388. FLOW ANALYSIS OF HUMAN MALIGNANT LYMPHOID CELLS

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This study represents an extension of our original project to evaluate flow microfluorometry (FMF) as a rapid and quantitative method for diagnosis, classification and monitoring of human lymphomas and leukemias. We have already shown that by FMF analysis, nearly 90% of Hodgkin's lymphomas can be distinguished from non-neoplastic tissues. Furthermore, preliminary clinical studies in lymphomas have demonstrated an excellent correlation between the percentage of cells in the S phase of the cell cycle as determined by FMF and the clinical course of the disease. These studies indicate that FMF may become an objective method in the diagnosis and classification of human lymphoid neoplasms, potentially more accurate than conventional morphology. In the present study, we will extend these observations to a larger number of patients with malignant lymphoid disease. We will emphasize the quantitation of S cells and the detection of abnormal cellular DNA content and light scatter emissions both singly and by simultaneous correlated analysis. The study of human B cell lymphomas by fluorescent beads coated with anti-light chain or anti-idiotypic antibodies to label selectively neoplastic B cells will allow the analysis of cellular DNA of malignant and non-malignant subpopulations obtained from the same sample. Response to therapy will be correlated with changes in the FMF histograms of the neoplastic samples obtained by needle aspirate or biopsy prior to and following administration of anti-neoplastic therapy. The late effects of anti-neoplastic therapy on DNA content in normal cells will be studied in lymphocytes of treated non-leukemic patients who are currently in remission.

389. CUTANEOUS T-CELL LYMPHOMAS

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Ultrastructural study of 18 skin biopsies from 18 patients with benign chronic skin diseases revealed that moderately convoluted lymphocytes (MDCL) occurred singly and were not observed in clusters within the dermal cellular infiltrates. In contrast, ultrastructural study of 30 skin biopsies from 20 patients with cutaneous T-cell lymphomas (CTCL) revealed the presence of clustered MDCL in dermal cellular infiltrates of 13 skin biopsies. Seven lymph node biopsies (LN) from seven patients not having CTCL were studied ultrastructurally. Three LN had dermatopathic lymphadenitis (DL) and four LN had benign reactive hyperplasia. Clustered MDCL were not observed in the paracortical areas of these seven LN. In contrast, clustered MDCL were found in the paracortical areas of 7 of 11 LN from 11 patients with CTCL. These LN also showed DL, but no diffuse lymphomatous infiltrate. Ultrastructural study of peripheral blood buffy coats (PBBC) from three normal control human beings revealed to MDCL constituted 4% or less of the small lymphocyte population. Ultrastructural study of 20 PBBC from 18 patients with CTCL revealed that MDCL constituted more than 4% of the small lymphocyte population in six PBBC from six patients. Ultrastructural study of a similar number of similar specimens is planned for the coming year.

390. EVALUATION OF SEVERAL PARAMETERS OF CELL GROWTH IN MYELOPROLIFERATIVE DISORDERS

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OBJECTIVE: To correlate several parameters at the onset and during the course of several myeloproliferative disorders in order to a) include further diagnostic criteria in the routine tests (standard laboratory, cytochemistry, morphology) when these are not sufficiently discriminatory for a precise classification, especially in acute leukemias; b) attempt to evaluate the results of therapy through changes in these parameters; c) identify significant changes that could be used as prognostic factors in these diseases.

APPROACH: The parameters that will be monitored to pursue the above mentioned objectives will include: a) Urinary colony stimulating factor (CSF) at the onset and during the course of independently diagnosed myeloproliferative disorders;

b) composition of single colonies grown in agar and recovered and specifically stained; c) karyotype analysis to detect changes during the course of the disease.

3. BRONCHOGENIC AND LUNG CANCER

391. KERATIN IMMUNOHISTOLOGY IN BRONCHOGENIC CARCINOMAS

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Precise histogenetic diagnosis of bronchogenic carcinomas is important for selection of the most effective therapy. The diagnostic category of large cell undifferentiated carcinoma (approximately 20% of our material) is at present of indeterminate origin and can be split into epidermoid and adenocarcinomas by means of electron microscopy only.

We propose to investigate the feasibility of immunohistological demonstration of keratin precursors to identify the poorly differentiated epidermoid carcinomas among this group. All bronchopulmonary neoplasms newly diagnosed at Sunnybrook Medical Centre during the two-year grant period and those collected during the two preceding years, will be stained with sequences of anti-keratin IgG (prepared in rabbits) followed by FITC conjugated and/or peroxidase labelled antirabbit IgG antiserum. Immunohistological results will be compared with classical histological and EM (when available) diagnosis. Over 250 bronchogenic carcinomas (including at least 50 large cell undifferentiated types) will be studied according to our present rates of new patients with bronchial neoplasms.

392. DETECTION AND LOCALIZATION OF BRONCHOGENIC CARCINOMA

Fontana, R. S., Mayo Foundation, 200 1st St. S.W., Rochester, Minnesota, 55901, United States of America

A reduction in personnel will occur in the following parts of this contract: recruiting of subjects, as well as accompanying costs for this part has been stopped; methodology for cytological examination has been well established, and the number of specimens that can be examined by any one cytology technician will probably be increased; the number of executive committee meetings will be reduced, thereby resulting in a reduction in travel requirements by project personnel; and the number of screeners will begin to drop, decline sharply in FY 1980, and cease altogether by FY 1981 or 1982. This depends on which month of the year the initial screening has occurred. After that, the screeners will be followed annually for five years to determine their status.

393. SPUTUM CYTOLOGY RELATED TO A FOUNDRY EXPOSURE

Simard, A., Band, P., Vauclair, R., Paquette, G., Saccomanno, G., Cole, P., Institut du Cancer de Montreal, Centre Hospitalier Notre Dame, 1560 Sherbrooke St. E., Montreal, Quebec, H2L 4M1, Canada

Some studies have shown an increased risk of lung cancer among foundry workers. Sputum cytology among uranium miners has shown cellular changes ranging from mild, moderate and marked atypia to cancer in-situ and invasive cancer. Sputum cytology will be undertaken in 700 foundry workers, using the technique of Saccomanno. Results will be related to duration of exposure in the foundry. Information on age, date of entry into the industry, work history, and smoking history will be obtained and person-years at risk evaluated. Comparisons will be made with other exposed groups, namely uranium miners, asbestos workers and smokers.

394. PREDICTION OF LUNG CANCER RATES BY SPUTUM CYTOLOGY

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In assessing the lung cancer risk of a worker population, it is highly desirable to have an early prediction method so that excess risk can be detected early. For new chemicals suspected of being pulmonary carcinogens, sputum cytology may reduce by half or more the time needed to determine the existence of risk, this possibly saving many lives. Several sputum samples from about 15,000 uranium workers have been collected over a

period of 18 years. Using Dr. Saccomanno's classification, the samples will be analyzed to show correlation with age, tobacco smoking and radiation exposure. Persons with various grades of atypia will be followed to see how their sputum changes over the years, and to determine subsequent lung cancer mortality associated with each class of atypia (up to 18 years). Multiple correlation analyses will be done (utilizing support from NCI). These analyses will permit assessment of the use of sputum cytology as a survey tool assessing the lung cancer risk of a population before lung cancer has appeared.

395. EFFECT OF EARLY DETECTION AND TREATMENT ON SURVIVAL FROM LUNG CANCER

Melamed, M. R., Flehinger, B. J., Miller, D. G., Martini, N., Zaman, M., Memorial Sloan Kettering Cancer Center, Dept. of Pathology, 1275 York Ave., New York, New York, 10021, United States of America

This study was undertaken to determine whether systematic, periodic examinations of sputum cytology, supplementing chest x-ray, could lead to earlier detection of lung cancer in a high risk group and to a reduction in death rate. The population chosen for study is volunteer men, in the New York metropolitan area, over the age of 45 years, who smoke more than one pack of cigarettes daily. A total of 11,000 men will be recruited into the study and all will have posteroanterior and lateral chest x-rays annually; approximately half will also have sputum cytology examinations at four-month intervals. The study is expected to require 5-10 years, and is being conducted in cooperation with the Mayo Clinic Foundation and Johns Hopkins University where additional populations are being surveyed in the same fashion.

396. DETECTION AND LOCALIZATION OF EARLY LUNG CANCER

Fontana, R. S., Sanderson, D. R., Pirolo, P. C., Williams, D. E., Cortese, D. A., Muhm, J. R., Miller, W. E., Woolner, L. B., Farrow, G. M., Taylor, W. F., Bernatz, P. E., Payne, W. S., Mayo Clinic, Dept. of Thoracic Diseases & Internal Medicine, 200 1st St. S.W., Rochester, Minnesota, 55901, United States of America

This prospective study relates to diagnostic methods of early lung cancer and assessment of survival of cases so detected. 9,223 male Mayo clinic out-patients, aged 45 or over, smoking 20 or more cigarettes daily, with no history of respiratory cancer on entry into the Clinic are included. All had a life expectancy of at least five years and were judged capable of tolerating pulmonary resection. At a first screening using chest x-rays, 3-day sputum cytology tests and lung health questionnaires, 87 unsuspected lung cancers were found, 16 by cytology alone, 54 by x-ray alone and 17 by both. Radiographically occult cancers were localized by fiber-bronchoscopy. Subjects with a negative initial screen were randomized into a close surveillance group, re-screened every four months, and a standard surveillance control group re-screened once a year. Haematomorphyrin derivative and cryotherapy are being evaluated as techniques for mapping and treating carcinoma in situ. As of 1 October 1979 the 4-monthly surveillance group and the control group had been observed more than 20,000 man-years, and 213 new cases of respiratory tract cancer detected, 51 involving the upper airway and 162 the lungs. In the 4-monthly surveillance group there were 98 new lung cancers (12 detected cytologically only) and 41 lung cancer deaths. In the control group there were 57 new lung cancers and 45 lung cancer deaths, with a tendency toward more usual survival periods. So far survival in the close surveillance group is better than among controls but there is no significant decrease in total lung cancer death rate. Observation and analysis continue.

397. CELL TYPE DISTRIBUTION IN HISTOLOGICAL SPECIMENS OF LUNG CANCER

Geddes, M., Buiatti, E., Cariaggi, P., Senori Costantini, A., Galli, G., Santucci, M., Center for the Prevention of Social Diseases, Unit for Epidemiological Research in Environmental Toxicology, 171 Viale Alessandro Volta, 50131 Florence, Italy

There are data supporting the fact that the distribution of different cell types of lung cancer depends also on the source of the histological specimen. This information may be useful for avoiding biases caused by the data source in epidemiological studies on lung cancer and cell types. The purpose of the study is to revise cell-type diagnoses in about 2,000 cases of lung

cancer, obtained from the Institute of Pathology of Florence. The 2,000 lung cancer diagnoses are from bronchial biopsy and operation specimen series; in addition, 400 cases of lung cancer for which a cytological diagnosis using the Saccomanno method is also available will be classified in the same way. The study will also give information about the distribution of sex and age among the different histological types.

398. CYTOLOGICAL SCREENING FOR LUNG CANCER IN WORKERS EXPOSED TO HEXAVALENT CHROMIUM IN A CHEMICAL PLANT

Herivan, R. M., Ministry of Chemical Industry, Labour Protection & Hygiene Centre, Lab of Cytology, 59 Pop de Basesti, 73244 Bucharest 3, Romania

The object of the study is to monitor 50 workers exposed to hexavalent chromium in a department in which chromium catalyst is manufactured, using cytological screening of sputum for the early detection of lung cancer as well as of epithelial disorders which may precede the development of a tumor. Annual cytological screening of sputum collected for three consecutive mornings and kept in Eskenasy fixative solution is carried out. Paraffin was added and the sputum dyed with HE and Masson. Results are correlated with age, sex, smoking habits, length of exposure, pulmonary antecedents, and exposure levels.

399. HUMAN LUNG NEOPLASMS - APPLICATIONS OF ENZYME PATHOLOGY

Greengard, O., City University of New York, Mount Sinai School of Medicine, Dept. of Pediatrics, 1 Gustave L. Levy Pl., New York, New York, 10029, United States of America

The purpose of this study on human lung is to increase the sensitivity of diagnosis of neoplastic transformation and to advance our ability to predict the kind of chemotherapeutic agents to which the different types of lung cancers would respond best. Neoplasms and tumor-free areas of resected lobes or surgical biopsy samples will be subjected to detailed histological examination. In aliquots of the same samples, and also in human fetal lung, we shall investigate the biochemical equipment which regulates the metabolic and proliferative potential of the different pulmonary tissues. Sets of enzymes whose amount or isozyme pattern can characterize different classes of pulmonary neoplasms and the degrees of differentiation of individual tumors will be identified. We plan to show that enzyme pathology can introduce sensitive, objective, quantitative criteria into the cytological characterization of hyperplastic foci and the early detection of precancerous lesions.

BIBLIOGRAPHIC REFERENCES: Greengard, O. Detection of Extrahepatic Cancer by Alterations in Hepatic Functions. *Biochem. Pharmacol.* 28: 2569-2572, 1979.

400. STUDY OF EARLY DETECTION OF CANCER OF THE LUNGS

Frost, J. K., Johns Hopkins University, School of Medicine, Dept. of Pathology, 725 N. Wolfe St., Baltimore, Maryland, 21205, United States of America

A reduction in personnel will occur in the following parts of this contract: recruiting of subjects, as well as accompanying costs for this part, has been stopped; methodology for cytological examination has been established, and the number of specimens that can be examined by any one cytology technician will probably be increased; the number of executive committee meetings will be reduced, thereby resulting in a reduction in travel requirements by project personnel; and the number of screeners will begin to drop, decline sharply in FY 1980, and cease altogether by FY 1981 or 1982. This depends on what month of the year the initial screening had occurred. After that, the screeners will be followed annually for five years, to determine their status.

401. ASSESSMENT OF MALIGNANCY GRADE BY QUANTITATIVE CYTOCHEMICAL METHODS

Caspersson, T. O., Auer, G., Konaka, C., Karolinska Sjukhuset, Dept. of Tumor Pathology, S10401 Stockholm 60, Sweden

In a retrospective study of fine-needle-aspiration biopsy material from 112 patients with primary mammary carcinoma the relationship between the type of the DNA-histogram and the patients' survival time has been investigated. The results show a distinct correlation between certain types of DNA-patterns and the prognosis for the patient. Further studies are in

progress using recent material in which other cytochemical parameters are also being investigated.

The different cell types appearing at different times during the development of experimentally induced bronchial carcinoma in dogs are being analyzed cytochemically. The tissue material is being provided by a Japanese group.

4. UROGENITAL CANCER

402. CHARACTERIZATION OF PROSTATIC TUMORS BY FLOW-CYTOFLUOROMETRIC DNA ANALYSIS IN ASPIRATION BIOPSIES

Tribukait, B., Anderson, L., Esposti, P., Ronstrom, L., Karolinska Institutet, Dept. of Medical Radiobiology, Solnavagen 1, Box 60400, S10401 Stockholm 60, Sweden

Quantitative DNA analysis has been used to characterize many different types of neoplasms. A correlation has been found between the degree of ploidy, cell differentiation and tumor aggressiveness. We have analyzed aspiration biopsies from various prostatic lesions with a flow-cytofluorometric technique. The results will be correlated with clinical and laboratory data in a follow-up study of untreated and treated cases of carcinoma of the prostate.

403. FLOW CYTOMETRIC ANALYSIS OF THE THERAPEUTIC RESPONSE OF THE PROSTATIC CARCINOMA

Irvine, G. L., Pollack, A., Bagwell, C. B., Block, N. L., U.S. Veterans Admin., Dept. of Medicine & Surgery, Medical Center, Surgical Service, 1201 Northwest 16th St., Miami, Florida, 33125, United States of America

PROJECT OBJECTIVES: (1) To apply the technique of flow cytometric DNA histogram analysis (FCM) towards the detection of prostatic carcinoma response to therapy. (2) To identify, separate, and characterize cells which respond to hormone therapy using FCM and hormone receptor analyses.

PLAN: The hormone dependent R3327 rat prostatic adenocarcinoma model is being used to test various FCM computer-based methods for detecting changes which result from endocrine manipulation of tumor growth. Also, the androgen and estrogen binding properties of R3327 cytosol and nuclear extracts are being characterized. Preliminary results show that endocrine manipulation (e.g. orchiectomy) causes a significant reduction in the number of androgen receptors which is reflected as a concomitant reduction in the proportion of hyperdiploid cells by FCM. The effects of diethylstilbestrol on androgen and estrogen receptors is being investigated. Also, cell separation techniques (e.g. sorting, elutriation) may allow for the identification of hormone dependent and independent tumor cells.

The above methods are being applied to human prostatic carcinoma tissue as well. Tumor tissue from transurethral-resection is being analyzed by FCM and this in turn compared to the histology of the specimen. Patients that have not been treated, are in the middle of estrogen therapy, and those who are in relapse are being studied.

404. THE CYTOLOGICAL DIAGNOSIS OF BLADDER TUMORS IN DYESTUFF WORKERS

Ishizu, S., Tokyo Womens Medical College, Dept. of Public Health, 10 Kawadacho Shinjuku-Ku, Tokyo 162, Tokyo, Japan

The production and use of benzidine and beta-naphthylamine in the dye industry of Japan were prohibited by law in 1971. However, there are still 4,037 workmen (including retired men) who have been exposed to carcinogenic amines, and risk developing a bladder tumor in the future. This study aims to perform continuous follow-up urine examination of the workers. For the early detection of bladder tumors, routine cystoscopy was considered to be quite unsuitable. The Papanicolaou smear technique of urine examination was introduced into the dye industry of Japan in 1961. A total of 4,037 men were screened first by wet smear and then by Papanicolaou smear. During the 22 years from 1955 to 1977, 192 cases of occupational bladder tumor were discovered; 15 out of 108 men with positive Papanicolaou smears have shown no tumors on cystoscopy; 7 men with negative Papanicolaou smears showed bladder tumors on cystoscopy (false negative); 47 out of 137 men with suspicious Papanicolaou smears had bladder tumors. Seventeen specimens obtained from the occupational bladder tumor patients were histopathologically examined. Of these 17 specimens, 8 showed

papillary cancer type, 6 showed an inverted proliferating tumor, and 3 were carcinoma in situ. This study continues.

405. CYTOLOGIC DETECTION OF BILHARZIAL BLADDER CARCINOMA

El Bolkalny, M. N., National Cancer Inst., *Kasr El-Aini St., Fom El-Khalig, Cairo, Egypt*

A demographic survey has been completed for 10 villages and farms, with a total population of 20,528 individuals. The high-risk group (farmers aged 20 years and above) constituted 25.4% of the entire population. So far, 4 cases of bladder carcinoma have been detected among 1332 persons at risk, a yield of 3/1000, and no tumors have been found among 2736 persons in the low-risk groups. Three tumors were squamous carcinomas and only one was transitional type. Two of the detected tumors were at an early stage (TIS and T1). One case of false positive cytologic diagnosis was made in this series. The overall frequency of bilharzia ova in urine was 12.5%, squamous metaplasia 7.4%, mild dysplasia 6.9%, and moderate to marked dysplasia 0.7%.

406. URINARY CYTOLOGICAL INVESTIGATIONS FOR DETECTION OF BLADDER CANCER AMONG RUBBER WORKERS EXPOSED TO PHENYL-2-NAPHTHYLAMINE

Herivan, R. M., Ministry of Chemical Industry, Labour Protection & Hygiene Centre, Cytology Laboratory, *59 Pop de Basesti, 73244 Bucharest 3, Romania*

The objective is to monitor the incidence of urinary bladder cancer in subjects exposed to phenyl-2-naphthylamine in the rubber industry. Cytological exfoliative screening with Giemsa stain and Papanicolaou's classification is used for early detection.

407. PLOIDY AND PROLIFERATION IN HUMAN BLADDER CARCINOMA AS MEASURED BY FLOW-CYTOFLUOROMETRIC DNA-ANALYSIS (ABBREV)

Tribukait, B., Edsmyr, F., Gustafson, H., Andersson, L. O., Esposti, P., Karolinska Institutet, Dept. of Medical Radiobiology, *Solnavagen 1, Box 60400, S10401 Stockholm 60, Sweden*

Quantitative flow microfluorometric DNA analysis provides possibilities for determining degree of ploidy and proliferation rate in tumor specimen and exfoliated tumor cells. The technique has been used in studying bladder neoplasms. A relationship between degree of ploidy and proliferation, and the histological picture and the clinical stage of bladder tumors has been found. The significance of these results for the development of the disease is analyzed in a follow-up study of 400 patients.

408. INVESTIGATION INTO THE USE OF MALIGNANT CELL CYTOLOGY SCREENING FOR BLADDER CANCER IN A CHEMICAL COMPANY

Cartwright, R. A., Gadian, T., Garland, J. B., Yorkshire Regional Cancer Org., *Leeds LS16 6QB, England, United Kingdom*

Routine screening has been undertaken by a chemical company since 1952. The firm had produced carcinogens but stopped production before that date. The aim of the study is to assess the survival of workmen diagnosed as having bladder cancer after screening as contrasted to those presenting symptomatically. All cases of bladder cancer in workmen have been identified and details of screening recorded as well as their outcome. Cases not industrial in origin but treated contemporaneously have been identified elsewhere in the city and used as a matching group in terms of treatment regimens. The study awaits a few details of follow-up before completion.

409. DUPONT CHAMBERS WORKS BLADDER CANCER SCREENING PROGRAM

Vogler, W. J., E.I. du Pont de Nemours & Co. Inc., Research & Development Laboratory, *Deepwater, New Jersey, 08023, United States of America*

OBJECTIVE: Document as thoroughly as possible the operations and results of the DuPont Bladder Cancer Screening Program so as to aid in the evaluation of such screening at the present time. Such documentation of a screening program involves a description of the population which is screened, a description of the screening test and its application to the population, and a description of the results of the interaction between

the test and the population in terms of incidence and mortality outcome measures. The product of this investigation will be in two parts. The first is a detailed study of exposure-natural history relationships and estimation of latent period. The second is a characterization of the potential for urinary cytology to detect early bladder cancer and a qualification of the survival experience of cases detected both within and outside the screening program.

410. IMMUNODIAGNOSTIC ASSAY OF BLADDER CARCINOMA

Monaco, A. P., Gozzo, J. J., De Fazio, S. R., New England Interstate Water Pollution Control Commission, *607 Boylston St., Boston, Massachusetts, 02106, United States of America*

An assay is needed for the diagnosis of bladder cancer that is sensitive, accurate and suitable for screening high risk populations. Current diagnostic methods involve patient risk and discomfort, are unsuitable for detection of low grade neoplasms or lack specificity. Our previous work suggests that there are tumor-associated antigens (TAA) in the urine of bladder cancer patients and that detection of these antigens in urine has potential for diagnosis of bladder cancer, even low grade bladder neoplasms. Although one of these antigens is also found in urine of patients with urinary tract infections, other antigens appear to be more specific to bladder cancer.

We propose to continue the biochemical isolation and characterization of these antigens to prepare specific antisera. These antisera will be used to continue the development of an enzyme-linked immunoassay (microELISA), suitable for use in a clinical laboratory. They will also be applied to a study of exfoliated tumor cells in urine by immunofluorescence. Finally, we will quantitate these antigens in the urine of bladder cancer patients and suitable control populations and perform an extended, multi-hospital study to determine the usefulness of our assay in the detection of bladder cancer.

BIBLIOGRAPHIC REFERENCES: O'Brien, P., Gozzo, J.J., Cronin, W. and Monaco, A.P., Qualitative analysis of proteinuria associated with bladder cancer. *Invest. Urol.* 17:28-32, 1979. O'Brien, P., Gozzo, J.J., Cronin, W.J., and Monaco, A.P. Urinary proteins as biological markers: Bladder cancer diagnosis versus urinary tract infection. *J. of Urology* (1979), in press.

411. AUTOMATION OF BLADDER CANCER CYTOLOGY BY TICAS

Koss, L. G., Schreiber, K., Moussouris, H. F., Freed, S. Z., Montefiore Hospital & Medical Center, Dept. of Pathology, *111 E. 210th St., Bronx, New York, 10467, United States of America*

Further progress is reported in the system for objective characterization of cells in the human urinary sediment by computer. In addition to 4 previous cell classes for which excellent identifying algorithms have been established, several additional groups of cells were studied with satisfactory discrimination. Initial application of the computer algorithms to visually selected well preserved urothelial cells has been shown to be of diagnostic value in 12 patients. A diagnostic analysis of 13 additional patients is in progress on a small laboratory computer and preliminary results are encouraging. A hierarchical analysis of 8 different classes of urothelial cells documented that the cells can be separated into 2 groups, one of which yielded data of diagnostic value. A new method for processing urinary sediment for high resolution scanning has been developed. Further work is in progress in programming small laboratory computer for cell analysis and first attempts at automated full scale analysis of cells in the urinary sediment are planned. The project explores the potential of currently available technology for the cytologic diagnosis of bladder cancer by computer.

BIBLIOGRAPHIC REFERENCES: Koss, L.G.: Urinary cytology - the subjective diagnostic clues and their evaluation by computer. *Anal. Quant. Cytol.* 1: 202-206, 1979.

412. URINARY CYTOLOGY - CYTOPATHOLOGICAL CORRELATION

Vargas, C., Castellanos, A., Guevara, S. I., Instituto Nacional de Cancerologia, Grupo de Urologia, *Apartado Aereo #17.158, Bogota, DE, Colombia*

OBJECTIVE: To identify the diagnostic value of cytological study of exfoliated material in urine.

MATERIAL: Exfoliated cells from the urine of patients diagnosed as having both benign and malignant lesions of the

kidney, bladder and urethra or of the uterus, ovary, vulva and rectum.

METHODS: a) Centrifugation of the urine, fixation of the cells and Papanicolaou staining; cytological study. b) Analytical study of clinical histories of patients whose urinary sediment has previously been subjected to cytological examination. (Translated)

413. SCANNING ELECTRON MICROSCOPY DETECTION OF BLADDER CANCER (HUMAN)

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This is part of a broader project. A summary of this subproject is not available.

414. AUTOMATED URINARY CYTOLOGY FOR CANCER DETECTION

Melamed, M. R., Darzynkiewicz, Z., Sharpless, T. K., Kapuscinski, J., Klein, F. A., Whitmore, W. F., Memorial Hospital for Cancer & Allied Diseases, Dept. of Pathology, 1275 York Ave., New York, New York, 10021, United States of America

The purpose of this program is to develop flow cytometry instrumentation and cytochemical techniques for automated, quantitative cytologic examinations of exfoliated epithelium of the urinary bladder, lymphocytes from lymph nodes and peripheral blood and tissue culture cells. With our present computer-interfaced flow cytometry instrument system and specially developed fluorescence acridine orange differential staining, we are able to measure DNA, RNA, chromatin structure and nuclear and cellular diameters of cells at rates of several hundred cells per second. In preliminary clinical studies carcinomas of the bladder have been successfully identified from these measurements on exfoliated epithelium in irrigation specimens.

BIBLIOGRAPHIC REFERENCES: Collste, L.G., Darzynkiewicz, Z., Traganos, F., Sharpless, T.K., Sogani, P., Grabstald, H., Whitmore, W.F., Jr. and Melamed, M.R.: Flow cytometry in bladder cancer detection and evaluation using acridine orange metachromatic nucleic acid staining of irrigation cytology specimens. *J. Urol.* 123:478-485, 1980.

415. AUTOMATED DETECTION OF UROTHELIAL TUMOR CELLS

Ploem, J. S., deVoogt, H. J., Brussee, J. A., deLeeuw, M., Rijksuniversiteit te Leiden, Sylvius Labs., Dept. of Histochemistry & Cytochemistry, Wassenaarseweg 72, P.O. Box 9303, 2300 RA Leyden, Netherlands

The objective of our research is the automation and standardization of the morphological diagnosis of urinary cell sediments. The research was started with a semi-automated investigation of urinary cell preparations stained with a fluorescent bicolor method (acriflavine-Feulgen-stilbene). These preparations were screened manually for cells which on visual inspection appeared to have a high DNA content. The fluorescence of these nuclei was subsequently measured. This procedure allowed an evaluation of the usefulness of cells with a high DNA content as a prescreening criterion and as a diagnostic feature. The results in about 100 urinary cell preparations showed that all grade II, III and IV tumors did contain cells with a high DNA content. These cells were rarely detected in some benign conditions such as inflammation and calculi, but never in normal urine from control persons. For automated screening a television based system is used. Programming and construction of the LEYTAS (Leyden Television Analysis System) image analysis computer were performed in co-operation with the Centre Morphologique et Mathematique, Fontainebleau, and Leitz, Germany. Preliminary results show a very effective separation between negative and positive preparations and detection with an extremely low number of artifacts of all positive preparations. Further research will be concentrated on implementing more advanced nuclear parameters programs (e.g., details of chromatin distribution within the nucleus) in LEYTAS. Also a larger field trial will be carried out.

416. AUTOMATED CANCER CELL DIAGNOSIS BY THE TICAS METHOD

Wied, G. L., Bartels, P. H., Bibbo, M., Yamauchi, K., University of Chicago, Pritzker School of Medicine, Dept. of Obstetrics &

Gynecology, 5801 S. Ellis Ave., Chicago, Illinois, 60637, United States of America

Computer analysis of cytophotometric data is used to classify automatically cells from clinical cytologic samples. The objective assessment of single cells is extended to a quantitative characterization of the clinical sample, so that a diagnostic evaluation of the patients can be made. Recent work has concentrated on the search for clues to the presence of neoplastic disease even when clinical sampling failed to procure frank cancer cells. It appears that even the normal intermediate cells from the ectocervix carry diagnostic marker features which allow a correct patient diagnosis for the presence of malignant disease without ever considering the cancer cells. Since this finding has significant implications for strategies in the prescreening for cervical cancer, and for the biological interpretation of processes within the cervical epithelium, concomitant with the occurrence of malignant lesions, descriptive statistics for the marker features, and their discriminatory potential have been collected.

BIBLIOGRAPHIC REFERENCES: Bibbo, M., Bartels, P.H., Sychra, J.J., Wied, G.L.: Chromatin appearance in intermediate cells from patients with uterine cancer. *Acta Cytol.* 25:23-28, 1981. Wied, G.L., Bibbo, M., Bartels, P.H.: Computer analysis of microscopic images: Applications in cytopathology. In *Pathology Annual*, 1981. Edited by S.S. Sommers, P.P. Rosen. New York, Appleton-Century Crofts (in press).

5. GASTROINTESTINAL CANCER

417. CYTOCHEMICAL STUDY OF GASTRIC BRUSHINGS AND EFFUSIONS USING FLUORESCAMINE STAINING FOR DETECTION OF MALIGNANCY

Sukumaran, M. S., Majhi, U., Rajalakshmi, K. R., Cancer Inst., Dept. of Pathology, Adyar, Madras 600020, Madras, India

OBJECTIVE: The differential diagnosis of early gastric carcinoma from lesions such as atrophic gastritis and intestinal metaplasia is difficult. Identification of malignant cells from abnormal mesothelial cells also is a problem to the cytologist. Fluorescamine has been used successfully in the differentiation of cancer cells from the normal counterparts in breast, intestinal and thyroid tumors. We intend to study the usefulness of fluorescamine staining technique in: 1) Differentiating the malignant cells from cells showing benign changes in the gastric brushing specimen. 2) In differentiating tumor cells from mesothelial cells.

APPROACH: 1) Gastric brushing specimens are collected. One set is stained by the fluorescamine stain and another by 'Pap' stain. The findings will be correlated with clinical and histological diagnosis. 2) Pleural and peritoneal effusions will be collected and an equal number of slides will be processed by fluorescamine and Pap techniques. The findings will be correlated with the clinical and histological diagnosis. The study has just commenced.

418. FREQUENCY OF INTESTINAL METAPLASIA WITH COLONIC TYPE CHARACTERISTICS IN THE STOMACH OF PATIENTS WITH GASTRIC CARCINOMA AND BENIGN LESIONS

Sipponen, P. I., Seppälä, K., Varis, K. S., Kekki, M., Siurala, M., Jorvi Hospital, Dept. of Pathology, 02740 Espoo 74, Finland

The investigation is designed to clarify the frequency of intestinal metaplasia (IM) with histochemical colonic-type characteristics in the stomach of asymptomatic subjects and of patients with gastric lesions of different types. The aim is to find out whether the occurrence of IM with colonic-like characteristics is associated with gastric carcinoma or with lesions considered to be pre-malignant. The study is performed by using histochemical staining methods, known to be characteristic to the colonic mucosa and mucosubstances, on mucosa specimens obtained by gastroscopy. 125 patients with verified gastric carcinoma and 301 first-degree relatives, 62 patients with pernicious anemia, and 183 first-degree relatives, 406 consecutive outpatients and 358 controls matched from a large Finnish population study are being examined. The investigation will continue as a follow-up study in patient groups selected according to the presence or absence of IM with colonic type characteristics in the tissue samples.

419. PHENOTYPIC EXPRESSIONS OF INHERITED DISEASE LEADING TO GASTROINTESTINAL CANCER

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In this project, indices defining the stages of preneoplasia are analyzed in individuals highly susceptible to the development of colon cancer. Patients are studied who have increased susceptibility to gastrointestinal cancer due to inherited adenomatosis of the colon and rectum and related diseases. After clinical examinations, biopsies and cytologic washings of colonic mucosa are studied to detect increasing indices of neoplastic transformation. Skin biopsies are taken from the upper arm of the patients, cutaneous cells cultured, and studied for changes in growth characteristics in various media and under the influence of growth factors. Blood specimens are taken for analysis of cell mediated humoral immunologic parameters. The ability of agents to induce transformation of the patients' cells are analyzed. Individuals with inherited adenomatosis and those in lesser risk categories are classified by phenotype to identify heightened degrees of susceptibility to neoplasia.

420. GASTRIC CANCER - SCREENING AND ETIOLOGY

Monteiro, J. G., Oliveira, M. G., Romazinho, J. M., Gouveia, A. P., Oliveira, M. R., Torres, M. L., Pragana, M. L., Universidade de Coimbra, Faculdade de Medicina de Coimbra, Centro de Gastroenterologia, Rua Larga, Coimbra, Portugal

The study aims to (1) screen unsuspected gastric cancer, and (2) investigate possible etiological factors in the diet. A screening program, in which 1,500 individuals will be screened, in the Guarda district in Portugal, using radiological and endoscopic techniques and clinical examination, has yielded seven cases per 1,000 examined. If a lesion is found, cytological and biopsy examinations are carried out. It is intended to establish a control group at a later date, composed of persons of the same age-group (45 plus) from a district of low incidence of gastric cancer. Polycyclic hydrocarbons in the food usually eaten by the patients have been measured.

421. LIGHT AND ELECTRON MICROSCOPE HISTOCHEMISTRY OF EPITHELIAL MUCINS OF COLONIC CANCERS

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OBJECTIVE: The application of new light and electron microscopical histochemical methodology to colonic cancers in an attempt to relate specific changes in epithelial mucins to diagnosis, prognosis and carcinogenesis.

APPROACH: Our investigation has shown (J. Clin. Path. 30, 1056, 1977) that our light microscope histochemical methods show a definite difference in staining characteristics (and therefore the chemistry) between the epithelial mucins of normal and well and poorly differentiated adenocarcinomas and benign and malignant villous lesions in the colon. Furthermore, we have been able to identify metastases from colonic cancers (J. Clin. Path. 28, 656, 1975). This methodology is based upon the identification (both chemically and histochemically) of various types of O-acetylated sialic acids.

PROGRESS: We are hoping to develop a 'Pap test,' based upon swabbing the rectal and colonic mucosa, for the early control of adenocarcinoma of the colon.

422. SIZE AND LOCATION OF ADENOMATOUS LESIONS OF THE COLON AND RECTUM

Auerbach, O., Hammond, E. C., Garfinkel, L., Frasca, J., Rickert, R., U.S. Veterans Admin., Dept. of Medicine & Surgery, Medical Center, Senior Medical Investigator's Laboratory, Tremont Ave. & S. Centre St., East Orange, New Jersey, 07019, United States of America

In order to shed light on the frequency of undiagnosed primary colon-rectum cancers at autopsy a large number of colon specimens from hospitals in New Jersey, New York State, Hawaii, Puerto Rico, and London is being collected to observe the location of polyps and malignancies found in the colon and to determine if atypical lesions or carcinoma in-situ can be

found in a polyp. Since the start of collection, about seven years ago, 2,886 colon-rectum specimens have been accumulated. The colons are fixed in formalin, cleaned and examined grossly for evidence of neoplastic lesions and mapped according to their location in the bowel. The lesions are removed and prepared for microscopic examination. The remaining colon is embedded in paraffin and sectioned for later examination. Efforts have been concentrated on the 518 cases in which the colon-rectum was completely removed including the anal skin. The lesions were examined microscopically and the size, location, and type of mucosal lesions recorded. A paper reporting the results of this investigation was published in *Cancer* 43:1847-1857, 1979. Adenomas occurred in 50% of the cases. Most of them were small adenomatous polyps less than 10 mm in diameter. 19 clinically unsuspected cancers were found. All were found in adenomas. The results suggest that the vast majority of adenomatous polyps do not undergo malignant changes. The study continues.

423. EPIDEMIOLOGY, HISTOCHEMICAL AND BIOCHEMICAL FEATURES OF COLON CANCER PRECURSORS

Simon, L., Balogh, J., Schmidt, I., Kenez, P., Vesztegombi, Z., Tolna County Hospital, Dept. of Gastroenterology, Beri-Balogh Adam 7/23, P.O.B 85, 7101 Szekszard, Hungary

Previous morphological, biochemical and histochemical studies were performed to prove the existence of two types of chronic non-specific colitis. The genetic determination of the first type of colitis was hypothesized, and some biochemical and histochemical differential diagnostic signs established. The aim of this study is to search for correlations between these two types of chronic colitis and cancer of the colon. The incidence of colorectal polyps and colon cancer will be determined in three groups of patients, with different ethnic origins, but living in the same geographical areas and cultural conditions in a County of Hungary.

424. FOLLOW-UP OF COLO-RECTAL CANCER PATIENTS

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46 patients were entered into a prospective study in order to determine the significant clinical importance of the usual routine diagnostic tests for follow-up of patients with colorectal cancer. The protocol consisted of: (1) admission to the hospital; (2) history and physical examination; (3) chest x-ray; (4) barium enema; (5) liver scan; (6) hemoglobin and hematocrit; (7) alkaline phosphatase; (8) carcinoembryonic antigen; (CEA); (9) alpha-fetoprotein; (10) studies of occult blood in stools; (11) proctoscopy and; (12) colonoscopy. Results of the study revealed chest x-rays should be continued and obtained once annually unless otherwise indicated. Barium enema and proctoscopy can be replaced by an adequately performed total colonoscopy. Liver scanning need not be obtained unless the patient has an elevated CEA titer, elevated alkaline phosphatase, or an exploratory laparotomy is contemplated. Of the laboratory studies obtained, the CEA titer offers the most promising information. CEA and alkaline phosphatase determinations are complementary. Alpha-fetoprotein has no value in patients being followed for colorectal cancer.

425. COLORECTAL CANCER IN INFLAMMATORY BOWEL DISEASES

Gennaro, M. N., Di Silverio, C., Terrizzi, A., Università degli Studi di Genova, Centro Polidisciplinare Malattie del Colon-Retto, Genoa, Italy

OBJECTIVES: Early diagnosis of colorectal cancer in inflammatory bowel disease. Identification of high risk subgroups by means of histological and cytological studies.

APPROACH: Of the techniques used, we want to test intrabioptic CEA and classic vital colorations for detection of dysplastic areas. The follow-up includes double contrast Rx enema, hemocult (including a study of anaerobic flora) ematochemical tests.

6. OTHER CANCERS

426. ASSESSMENT OF ASPIRATION CYTOPATHOLOGY AS A TOOL IN THE RAPID DIAGNOSIS OF NEOPLASMS

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OBJECTIVE: The present work is aimed at finding out the efficacy of needle aspiration biopsy in the diagnosis of palpable lumps.

APPROACH: Patients presenting with palpable lumps are first clinically evaluated, then the aspiration biopsy is carried out by a simplified technique using a fine (18-20 gauge) hypodermic needle, fitted to an all glass (20 cc) syringe. No anesthesia is given. The aspirate is spread over one or more clean glass slides (depending upon the amount of material). The smears are fixed in 95% alcohol in wet state and stained by routine hematoxylin and eosin stain. Simultaneously, conventional histopathological examination is done of the open or excisional biopsy of the corresponding lesions, wherever required and feasible. The results of both the techniques will be combined later on.

PROGRESS: To date, 290 tumors have been aspirated for cytological examination. The results are encouraging (84.2%) in cases of malignant epithelial tumors, where as sclerosing mesenchymal tumors pose a small problem in a proper cytodiagnosis as a positive cytohistopathological correlation was observed in only 60% of the instances. Further studies are in progress.

427. DIFFERENTIAL DIAGNOSIS OF BORDERLINE LESIONS - MALIGNANT AND REACTIVE - USING MICROPHOTOMETRY - COMPARISON OF NUCLEAR DNA CONTENT

Tanaka, N., Ueno, T., Chiba Cancer Center Research Inst., Dept. of Pathology, 666-2, Nitona-Cho, Chiba 280, Chiba, Japan

OBJECTIVE: Morphological differential diagnosis of borderline lesions distinguishing between malignant and reactive is often difficult in practice. Comparison of nuclear DNA contents of such lesions appears to provide a possible answer to the problem of discrimination between malignant and benign or reactive lesions.

APPROACH: Various kinds of lesions which are not easily classifiable as malignant or benign or reactive, such as dysplasia, mastopathia, lymphomas, papillomas or Grade I carcinoma of various sites, etc. are collected and examined using Olympus MMSP microphotometer. The specimens, both smear preparations and histology sections of the same cases, were tested using Feulgen reaction. Histograms of nuclear DNA content were provided and compared with those of normal DNAs contents.

PROGRESS: Data analysis continues.

428. EVALUATION OF CYTOLOGICAL BONE MARROW CRITERIA IN DIFFERENTIAL DIAGNOSIS OF MM AND BMG (ABBREV)

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Reliable differential diagnostic criteria between multiple myeloma (MM) and benign monoclonal gammopathy (BMG) are urgently needed, as has been shown in a previous work.

In the proposed project we plan to study in detail cytological criteria applied to bone marrow samples obtained from patients with well-defined MM or BMG. Hopefully, results obtained will improve diagnostic acumen in patients with monoclonal gammaglobulins.

429. DIAGNOSIS OF INTRACRANIAL TUMORS BY CYTOLOGY OF CEREBROSPINAL FLUID

Parulkar, G. B., Chitale, A. R., Nagpal, R. D., Gandhi, V. A., King Edward VII Memorial Hospital, Dept. of Surgery, Parel 400012, Bombay, India

OBJECTIVE: To study cerebrospinal fluids cytologically for space-occupying lesions.

APPROACH: The cerebrospinal fluids obtained by ventricular and lumbar puncture were examined during a period of 6 months. Cystic fluids were also included in this study. Smears were prepared, stained by hematoxylin-eosin method and the

results reported immediately. Excluding the unsatisfactory preparations, the accuracy rate assessed in 150 cases was 92%.

430. STUDIES OF NASAL CANCER IN WORKERS EXPOSED TO NICKEL AND DEVELOPMENT OF METHODS FOR SCREENING AND EARLY DIAGNOSIS (ABBREV)

Boysen, M. E., Reith, A. K., Norsk Hydros Inst. for Cancer Research, Laboratory of Electron Microscopy, Montebello, Oslo 3, Norway

OBJECTIVE: Histopathological follow-up study of known cases and screening for new cases of precancerous lesions by light microscopy (LM). Characterization of normal, metaplastic, dysplastic (precancerous) and cancerous lesions of the nasal mucosa by means of scanning and transmission electron microscopy (SEM, TEM), morphometry, autoradiography (cell kinetics), and development of cytological screening methods for cancerous and precancerous lesions and an experimental model using rats for upper airway carcinogenesis.

APPROACH: Biopsies from the nasal mucosa of workers in a nickel refinery were plastic-embedded for histopathology and morphometry. A method was introduced for characterization of large mucosal areas by SEM and, thereafter, areas of interest were sectioned for comparison with LM and TEM. Cytological samples from the regions adjacent to the biopsy were taken with a special brush. A new model was developed for morphometric characterization of the different histopathological stages of nasal mucosa.

We will now develop an experimental model with rats for inhalation and installation of carcinogens to be used for comparison with the pathological alterations seen in human nasal mucosa. Furthermore, cytokinetic evaluation will be made by in vitro incubation in tritiated thymidine.

PROGRESS: The histopathological follow-up study has shown a marked decrease in the number of cases with dysplasia, probably as a consequence of the environmental conditions in the refinery in recent years. Our morphometric studies show that different histopathological stages can be characterized objectively (Pathology: Research and Practice, in press). The SEM studies have shown that the different stages are also reflected in different surface structures of cells at the luminal site.

431. HISTOMORPHOLOGIC STUDY OF BUCCAL CARCINOMA

Santana, J. C., Miranda, J. D., Instituto Superior de Ciencias Medicas de la Habana, Departamento de Patologia Bucal, Av. 100 No. 9522, 6/10 Rpto. Altahabana, Havana 8, Cuba

OBJECTIVE: To determine the histomorphologic and enzymatic characteristics of buccal carcinoma, comparing them with prognosis and clinical course.

METHOD: Topographical and anatomoclinical classification of carcinoma of the buccal mucosa, with particular emphasis on clinical stage; histomorphologic classification; enzymatic and classic histochemical study of histologic variations. Comparison of these findings with the capacity for dissemination and the prognostic and clinical evolution to a determined and close stage.

PROGRESS: Determination of the normal histochemical patterns of buccal mucosa and development of the techniques to be used.

432. EVALUATION OF DIAGNOSTIC PROCEDURES IN CARCINOMA OF THE ESOPHAGUS

Charan, A., Ravikant, Ramakant, Misra, N. C., King Georges Medical College, Dept. of Surgery, RH 302, Lucknow 226003, India

OBJECTIVES: 1) Investigate suspected cases of carcinoma of the esophagus by various investigative procedures. 2) To assess the relative efficacy of these diagnostic procedures in the diagnosis of carcinoma of the esophagus. 3) To assess: a) status of Lugol's Iodine Test by endoscopic staining followed by biopsy; b) value of routine bronchoscopy and bronchial lavage for cytology in cases of carcinoma of the esophagus for determining operability.

APPROACH: In each case, the following investigations will be done (cases admitted in Gandhi Memorial and Associated Hospital, Lucknow, will be taken up for the present study): 1) Roentgenological - simple, constant, double constant; 2) esophagoscopy; 3) cytology; 4) histopathology of biopsied tissue; 5)

Lugol's Iodine Staining of esophagus by endoscopic method; and 6) routine bronchoscopy.

433. CLASSIFICATION AND HISTOPATHOLOGICAL DIAGNOSIS OF LYMPHORETICULAR INFILTRATES IN THE SKIN

Hautzer, N. W., McCaughey, W. T., Canadian Tumour Reference Centre, 60 Ruskin Ave., Clinical Studies Unit Bldg., Ottawa, Ontario, K1Y 4M9, Canada

It is the primary intent of the study to improve the differential diagnosis and classification of lymphoreticular infiltrates with primary manifestation in the skin. This is attempted by: a) Retrospective evaluation of the histopathology on H - E stained section, b) correlation with the follow-up, c) investigation of possible immunoglobulin production by the immunoperoxidase PAP method, d) identification of T-cell by the use of anti-T-cell serum or indirectly by determination of acid-phosphatase and/or non-specific esterase staining patterns.

All investigations will be performed on paraffin sections only.

434. EXFOLIATIVE CYTOLOGY APPLIED TO HUMAN DIAGNOSTIC PROBLEMS AND RESEARCH PROBLEMS

Chu, E. W., DelVecchio, P. R., Byrne, S. D., Wood, T. A., Galito, L., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Cytopathology Section, 9000 Rockville Pike, Bethesda, Maryland, 20205, United States of America

OBJECTIVES: Provide complete diagnostic service in exfoliative, medical cytogenetics, and fine needle aspiration cytology. In addition, collaborate in various clinical research projects utilizing special techniques including special staining and tissue culture techniques.

BIBLIOGRAPHIC REFERENCES: Mills, S.R., Doppman, J.L., Head, G.L., Javadpour, N., Brennan, M.F., and Chu, E.W.: Transcatheter brush biopsy of intravenous tumor thrombi. *Radiology* 127: 667-670, 1978. Chu, E.W., Hanson, T.A., Goldman, J.M., and Robbins, J.: Study of cells in fine needle aspiration of thyroid gland. *Acta Cytol.* 23: 309-314, 1979.

435. DEVELOPMENT OF A CYTOMETRIC DATA BANK

Sodier, E., Guman, N., Hospital Interzonal Mariano Castex, Servicio de Patologia, Balcarce 900, 1650 San Martin, Buenos Aires, Argentina

OBJECTIVE: To register cytometric data (nuclear and cytoplasmic diameters and size and percentage of micro- and macronucleoli) and cytomorphological data on histologic sections and cytologic specimens from approximately 600 malignant and benign tumors, hyperplasias, normal controls, etc.

This will provide data for an Automated Cellular Diagnostic System, and, during its development, will allow study of detailed classification of malignant tumors, relationship between tumors and preneoplastic atypias, oncogenic processes, etc.

METHOD: An ocular micrometer and immersion oil objective (1875 x) will be used in determining measurements and cytologic characteristics. The data will be processed electronically to obtain the arithmetic mean, standard deviation, and standard error and variance; they will be verified with the Student Test and Analysis of Variance. The results will be recorded on perforated cards.

PROGRESS: Data are complete on 1) colon adenomas, 2) endometrial hyperplasias, 3) cervical dysplasias and carcinomas in situ, 4) transitional cell carcinomas, papillomas and hyperplasias of the bladder, 5) basal hyperplasias and bronchial metaplasias, 6) non-Hodgkin's lymphomas, 7) nevi and melanomas, 8) canalicular breast carcinomas, 9) endometrial adenocarcinomas, 10) prostatic adenocarcinomas, 11) gestational endometrial hyperplasias, 12) colon adenocarcinomas, 13) gallbladder adenocarcinomas, 14) gastric adenocarcinomas, 15) atypias in chronic gastritis, 16) hepatomas, 17) skin basal cell epitheliomas, and 18) invasive squamous cell carcinomas of uterine cervix.

436. NEUROCOMPUTER PROJECT

Cala, L. A., Mastaglia, F. L., Quinlan, M., University of Western Australia, School of Medicine, Dept. of Medical Statistics, Perth, W. Australia 6009, Australia

OBJECTIVE: To diagnose site and type of intracranial tumor by means of the memory core of a computer.

APPROACH: The clinical signs and symptoms were coded into numbers from look-up lists of patients with a proven tumor. All radiological data, surgical details, radiotherapy treatment programs, the EEG and static brain scan, and the histology and progress of the patient were similarly treated.

PROGRESS: As a pilot scheme, new cases were matched against the memory bank for clinical data and radiological data only. A very high rate of successful prediction was achieved. The mathematical basis has to be further tested when more cases are added to the bank. Preliminary subsidiary paper is in press dealing with information relating to a separate item that arose as the main work was in progress - namely, diagnosis of posterior fossa tumors using the plain skull radiograph alone. Unfortunately, normal data were not available so the work to obtain this was undertaken first using a grant from S.C.G. Hospital.

FUTURE: 1. Computerized axial tomography (EMI scanner) will be used to add further to the interrelating radiological and clinical data. 2. The natural history of various forms of hydrocephalus is to be studied with special emphasis on that mechanism operating in obstructive hydrocephalus of tumor.

437. PROGNOSTIC FACTORS AFFECTING 5-YEAR SURVIVAL IN BREAST CANCER

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Five hundred fifteen cases of breast cancer treated during the years 1968-1974 at the Oulu University Hospital, Department of Radiotherapy, were analyzed to establish those prognostic factors which can be useful in discerning a high risk group in which the probability of developing a recurrence of the disease is very considerable. The factors chosen for this purpose were histological typing of the primary tumor, histological grading of malignancy, nuclear grading, clinical T N M classification (tumor size, axillary nodal involvement and classification by stage), location of the tumor within the breast, menopausal status and method of treatment.

Axillary nodal involvement is shown to be the predominant factor and is of great value in the establishment of the prognosis, the five-year survival for those cases with negative axillary involvement being 81.9% compared with only 37.8% for those cases with positive axillary involvement. Size of the tumor is also of considerable significance in establishing the prognosis, as the relative survival rates at five years were 96% for T1 cases, 77% for T2 cases, 49.3% for T3 cases and only 19.1% for T4 cases. Classification by stage in which both the size of the tumor and nodal axillary involvement are taken into account also proved to be useful. The respective five-year relative survival rates were: Stage I 88%, Stage II 52.4%, Stage III 40.8% and Stage IV 9.1%.

Histological typing of the primary tumor as recommended in the Classification of Breast Tumors (WHO 1968) seems to be very useful, giving great accuracy and uniformity in the classification of the tumors. The histological grading used here for the cases of the ductal infiltrating type of carcinoma showed an adequate correlation with the prognosis. The five-year survival rates for the histological grades of malignancy were: Grade I 85.5%, Grade II 64.9% and Grade III 43.5%. Histological grading and clinical staging together are of greater accuracy in establishing the prognosis than either factor alone. Nuclear grading is a prognostic factor of only relative importance. The location of the tumor fails to emerge as a significant factor in this study, although a trend towards poorer prognosis was observed for tumors centrally located. (Text Abridged.)

438. INFLUENCE OF CONTRACEPTIVE PILLS ON THE MILK DUCT EPITHELIUM IN THE MAMMARY GLAND

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The aim is to explore the influence of the oral contraceptive pill on the breast by cytological examination of nipple fluid. An increased frequency of mild, moderate and severe dysplasia was found in such persons, particularly those with high gestagen levels, the effect being particularly noticeable for pills containing norethisterone acetate. Of 3,000 women who had taken the pill for more than four years, 45 (1.5%) had dysplasia. Of 36

women with dysplasia who had taken the pill, three-quarters contained norethisterone acetate, in contrast with 50% in a control group. This investigation is continuing to determine whether norethisterone acetate is responsible for dysplasia of the milk duct by comparing long-term users and control women.

439. ULTRASTRUCTURAL STUDIES OF BENIGN AND MALIGNANT HUMAN THYROID TUMORS

Johannessen, J. V., Sobrinho Simoes, M. A., Perzin, K. H., Gould, V. E., Tangen, K., Radiumhospitalet, Laboratory for Ultrastructural Pathology, Montebello, Oslo 3, Norway

OBJECTIVE: To determine whether we can separate the benign thyroid tumors derived from the follicular epithelium from the malignant ones, by using transmission and scanning electron microscopy in conjunction with morphometry and DNA flow cytophotometry.

APPROACH: I have, during the past 6 years, collected several hundred specimens from human thyroid tumors. Most of the specimens have been secured for histopathology, cytology, transmission electron microscopy, scanning electron microscopy as well as histochemistry and DNA flow cytophotometry. The investigation aims to clarify whether there are diagnostically useful differences in the benign and malignant follicular thyroid tumors, as a proper classification may sometimes be impossible by light microscopy when no metastases are present.

The investigation furthermore aims to determine whether the follicular areas of papillary thyroid carcinomas are similar to follicular carcinomas or papillary ones, to determine whether the classification into mixed follicular and papillary carcinomas of the thyroid should be sustained or omitted. This not only has prognostic implications but also epidemiological ones, as some investigators now classify mixed tumors as such, while others classify them as papillary carcinomas, while still others classify follicular tumors with ground glass nuclei as papillary carcinomas.

PROGRESS: The work on the material has already started and preliminary results have been published.

BIBLIOGRAPHIC REFERENCES: Johannessen, J.V., Gould, V.E., Jao, W.: The fine structure of human thyroid cancer. *Hum. Pathol.* 9, 385-400, 1978; and Gould, V.E., Johannessen, J. V. and Sobrinho Simoes, M.A.: The Thyroid Gland in Electron Microscopy in Human Medicine, Vol. 10, Endocrine Organs (ed. J.V. Johannessen), McGraw-Hill International Book Company, New York, 1980, in press.

440. COMPARISON OF THYROID GLAND HISTOLOGY IN SOUTHERN AND NORTHERN NORWAY

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The incidence of thyroid carcinoma is two to four times higher in northern Norway than in the south. Gross and microscopic studies are being made of the thyroid gland from 259 consecutive autopsies from each of two laboratories, one in the southern inland and one at the northern coast, searching for occult carcinomas and possibly associated changes.

441. EPIDEMIOLOGY AND HISTOLOGICAL AND ELECTRON-MICROSCOPIC DIAGNOSIS OF THYROID CANCER - IODINE PROPHYLAXIS

Hofstadter, F., Leitner, G., Ladurner, D., Leopold Franzens Universität Innsbruck, Pathologisches Institut, Muellerstr. 44, A6020 Innsbruck, Austria

OBJECTIVE: To study endemic goiter, particularly with regard to a systematic iodine prophylaxis by means of iodinating salt, established in 1960.

APPROACH AND PROGRESS: In the Swiss endemic goiter area an analogous salt prophylaxis was introduced about 30 years ago. In this tumor material some interesting changes in morphology and clinical behavior have been noticed, and the results published.

We revised our histological tumor material from the years 1952-1975 and classified the specimens according to the WHO schedule, using uniform criteria. The exact morphological diagnosis (including number of infiltrations of the tumour capsule and blood vessels) was correlated with the clinical course of the disease. The total number of reclassified carcinomas was 453.

Furthermore we studied the epidemiological criteria of these carcinomas (sex, age, incidence) in contrast to the 10423 non-malignant thyroid tumors. In order to determine the value

of diagnostic improvement influencing the morphological pattern of thyroid tumors, we studied the preoperative clinical findings of the non-malignant thyroid tumors (symptoms, radiology, scintigrams, cytology) and correlated these data with the morphological features (weight, macroscopy, histology). For selected years (before and after the introduction of iodine prophylaxis) we studied the lymphocytic infiltration of the thyroid specimens and found a severe increase in this alteration after iodine prophylaxis. To evaluate this dependency of the morphology and biology of thyroid neoplasms on the specific endocrine situation we studied 25 thyroid carcinomas by means of electron microscopy. In differentiated carcinomas we often found disorganization of cytoplasmic organelles such as hyperplasia or hypertrophy of a single organelle type (mitochondria, lysosomes, rough and smooth ER, cytoplasmic vesicles). These findings will allow us to study the physiologic function of the respective cytoplasmic organelles.

442. SCREENING FOR EARLY HEPATIC MALIGNANCIES IN ASIAN PATIENTS ON ORAL HORMONAL THERAPY

Oon, C. J., Chan, S. H., Tshakok, M., Chio, L. F., Singh, R., Ya, S. L., Lim, G. Y., Cheng, L. Y., Goh, K. T., Tan, K. L., University of Singapore, General Hospital, Dept. of Medicine, Singapore 3, Singapore

HBsAg positive and HBsAg negative patients on hormonal therapy are screened for early hepatic malignancies using multiple tumor markers e.g. AFP, Alpha anti-trypsin, Alpha acid-glycoprotein, Gamma GT, SGPT, SGOT. Positive tests are then followed-up clinically with radio-isotope liver scans using Technetium. It is estimated that 500 females and males will be included in the study.

443. MASSIVE DETECTION OF ORAL AND MAXILLOFACIAL CANCER

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OBJECTIVES: 1. To detect oral and maxillofacial malignant and pre-malignant lesions in the population of the metropolitan area of Santiago, Chile; 2. To put into operation a service for early diagnosis; 3. To offer these services to the public; 4. To evaluate the diagnostic methods used; 5. To obtain prevalence and incidence rates for these lesions; 6. To teach pre-graduate students at the Faculty of Odontology; 7. To register and follow-up detected cases; to refer patients to the appropriate treatment centers.

METHOD: 1. Promotion of self-examination of the oral cavity in the public; 2. Clinical examination; photography; toluidine-blue test; exfoliative cytology (specimen collection, processing and diagnosis of slides) and biopsy of suspicious lesions; 3. Tabulation and evaluation of results according to the International Classification of Diseases-Oncology.

444. DIAGNOSTIC CRITERIA AND TECHNIQUES FOR DETECTION OF ASYMPTOMATIC ORAL CARCINOMA - IDENTIFICATION OF HIGH-RISK GROUPS

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It appears that erythroplastic portions of asymptomatic oral squamous cell carcinoma stain positive with toluidine blue. Very few false positives are found if toluidine blue is used in conjunction with previously defined clinical criteria (erythroplasia). Suspicious oral lesions found during routine oral examinations are referred to the investigator. Lesions which persist for 10-14 days are described, photographed, and a demographic questionnaire is completed by a trained interviewer. A clinical diagnosis is made and the lesion is stained using toluidine blue. Results are recorded. A biopsy renders final pathological diagnosis. Demographic data are being evaluated to delineate what percentage of these patients with oral squamous cell carcinoma drink or smoke heavily, have developed or have had other primaries of the upper aerodigestive tract, including the mouth. It should be noted that these are for the most part independent squamous lesions. Additionally, recurrence and survival rate for patients with early asymptomatic lesions is being studied. A

control group is also being studied. All admissions to the hospital have a scheduled appointment for oral examination in the Dental Clinic. Patients scheduled each day (over 40 years of age) are interviewed and questioned. The only bias in selection is that patients who are too ill to attend the clinic are not taken or that patients are inadvertently missed for interview. Since the patients with early erythroplastic oral cancer are those who are examined in the same way, the controls are in the same population. A paper was published in *Cancer* 46: 758-63, 1980.

443. METHODS OF EARLY DIAGNOSIS OF CANCER OF THE MOUTH

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OBJECTIVE: To verify the effectiveness of non-traumatic, innocuous and simple techniques that could be used on a large scale in out-patient clinics for the early diagnosis and prevention of cancer of the mouth.

APPROACH: Select 40 mouth lesions including white, ulcerative and tumorous lesions. Perform Papanicolaou test, staining with orthotoluidine blue, photography before and after staining, and conduct a biopsy and anatomicopathologic examination of the lesion. Once these data are obtained the findings will be compared in order to determine the efficiency of the Papanicolaou test and orthotoluidine blue stain in distinguishing between benign lesions and those of varying degrees of dysplasia. (Translated)

446. CLINICAL USE OF NP-59

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This is a diagnostic test to differentiate adrenal adenoma from hyperplasia as a cause of adrenal hyperfunction. Whether medical or surgical treatment is indicated depends on this differentiation.

447. CYTOMORPHOLOGIC AND CYTOMORPHOMETRIC ANALYSIS OF CANCER CELLS OBTAINED IN FINE-NEEDLE ASPIRATES

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OBJECTIVE: Today's more differentiated treatment of malignant tumors requires prognostic instruments. Breast cancer patients may receive cytostatic drugs and additional radiotherapy if the tumor is highly aggressive whereas other patients can be offered a small surgical excision only and still be in a prognostically favorable group.

Conventional microscopic evaluation cannot give reliable information about prognostic factors - DNA measurements as well as morphometric determination of certain parameters can be correlated with mitotic activity, proliferation activity, and chromosomal aberrations consistent with a dedifferentiation of the tumor cells.

APPROACH: Tumor cells obtained using a fine-needle aspiration technique will be analyzed cytomorphometrically for DNA-content and definable morphologic parameters will be registered. Variations regarding form, size, chromatin pattern and texture, nucleolar size and distribution, eccentricity and cellular dissociation will be correlated with DNA-measurements and other quantitative measurements of the malignant cells. These parameters will be correlated with biological behavior estimated in extensive workup programs and standardized treatments and follow-up schemes.

448. DEVELOPMENT OF EARLY DIAGNOSIS OF CANCER OF THE PANCREAS BY ERCP AND RELATED PROCEDURES

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OBJECTIVE: Purpose of this study is to develop early diagnosis of cancer of the pancreas by ERCP and related procedures.

APPROACH: (a) Comparative study between pancreatogram and its histology in postmortem pancreatograms. (b) Anal-

ysis and interpretation of pancreatograms of small tumors in postmortem pancreatography. (c) Improvement and development of techniques in ERCP for detecting small malignant lesions in the pancreas; direct magnification during ERCP. (d) Study of cytology and biochemical analysis of pure pancreatic juice collected by cannulation. (e) Development and clinical use of screening methods for pancreatic cancer; assays of RNase, CEA and amylase in serum and pure pancreatic juice, and evaluation of combined screening of these tests.

PROGRESS: The branches and fine pancreatic ducts when opacified with contrast material can be studied more precisely after direct fourfold magnification during ERCP than by routine ERCP. Patients with pancreatic cancer showed significantly higher serum RNase activity than patients with chronic pancreatitis or gastric cancer and healthy subjects.

449. PANCREATIC CANCER EPIDEMIOLOGY - SOURCES OF CASE MISCLASSIFICATION

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Pancreatic cancer has always been difficult to diagnose, and even with improved diagnostic techniques is most often diagnosed after metastasis or at autopsy. In view of the poor diagnosis the search for risk factors and high risk populations becomes very important. Pancreatic cancer cases used in epidemiological studies come from three main sources; death certificates, hospital series, and tumour registries. The source of diagnosis may lead to misclassification. The yield from epidemiological studies will be of higher quality when cases are similar and definitely pancreatic cancer. The object is to identify the amount of misclassification that occurs, paying special attention to the type and source of histological confirmation and diagnostic evaluation for the years 1955-1979. The study will cover all cases of pancreatic cancer identified at three University hospitals in North Carolina. Cases will be identified from the hospital tumour registries and autopsy records. Information will be taken from review of the medical chart, autopsy reports, death certificates, and all diagnostic and pathology reports. For each case a comparison will be made of clinical diagnosis, autopsy diagnosis when present, and death certificate diagnosis. The misclassification identified will be related to symptoms and histological confirmation. The effect of misclassification based on sources of cases for epidemiology studies will be estimated. Some 1,000 cases and 1,500 gastric cancer charts will be reviewed to evaluate the relationship of changing diagnostic techniques as the source of pancreatic cancer misclassification.

C. OTHER STUDIES ON CYTOLOGICAL DIAGNOSIS OF CANCER

450. BIOPHYSICAL PROBES FOR AUTOMATED CYTOLOGY

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This project will continue to develop new fluorescent probes for DNA and RNA through the synthesis of novel fluorescent labeled polynucleotides and specific base binding fluorescent compounds.

PROGRESS: Abstracts: Approaches to the Histochemical Localization of m-RNA for Enzyme Synthesis, VI International Histochemistry and Cytochemistry Congress 1980, Brighton, England. Development of a New Electron-dense Fluorescent Acridine Dye (FeAPA) for Chromosome Staining. 31st Annual Histochemical Society Meeting, New Orleans, Louisiana.

Reprints: *Nucleic Acids Research*, Volume 8, No. 3 (1980). *J. Histochem. and Cytochem* 28:pp. 1032-1038 (1980).

451. CYTOLOGY AUTOMATION

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We will investigate the feasibility of flow cytometric analysis of DNA, RNA, protein, nucleolar antigen and cell volume to identify the presence of tumor cells in clinical material. The

relative sensitivity and specificity of automated cytology to detect tumor cells will be compared to standard light and electronmicroscopic evaluations. Furthermore, flow cytometric parameters will be sought that reflect the degree of tumor differentiation and grading, in order to provide a clinically useful classification in the areas of lung, bladder and prostate carcinomas and of malignant lymphomas. We will assess whether such cellular probes vary with the site of neoplastic disease (TNM) and thus provide quantitative cellular features determining the sites of metastatic spread. For human breast cancer, we will develop concurrent DNA/estrogen receptor (ER) flow cytometry to permit measurements of ER quantity on a per-tumor-cell basis and with regard to cell cycle position. The generated information will facilitate a more objective and quantitative means of cancer diagnosis and prognosis.

452. PURCHASE AND INITIAL OPERATION OF FLUORESCENCE ACTIVATED CELL SORTER FOR VARIOUS CANCER-RELATED STUDIES

Clark, A. M., Bradley, J., Burgoyne, L. A., Cant, Cox, Morley, Murray, Keighley, Finlay Jones, Martin, Zola, H., Flinders University of South Australia, Flinders University Cancer Research Unit, Bedford Park, S Australia 5042, Australia

The Cancer Research Unit of the Flinders University of South Australia is purchasing a fluorescence activated cell sorter for use in cancer-related studies by research workers in South Australia. The instrument has yet to be installed but a number of research projects are awaiting the arrival of the instrument. The sorter will be used by workers using animal tumor models to analyze the cellular composition of tumors and to prepare purified subpopulations of lymphocytes in order to further analyze their function in the immunological response to cancer. Studies using human malignant cells will be of two general types. Some studies will deal with the analysis of the nucleic acid content of cells which reflects their cell cycle antibodies. It is anticipated that some of these studies will lead to improved diagnostic examination of leukemic cells and samples from patients in remission to detect residual disease and to make an early diagnosis of relapse.

453. LATHANIDE FLUORESCENT STAINS

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The objective of the proposed research is to develop a series of fluorescent stains suitable for the simultaneous multiple staining of cytological specimens in flow systems. While these stains are intended primarily for use in automated cancer screening and detection, their usefulness extends to any area of cytology where multiple fluorescent labeling might be advantageous.

The key features of these novel stains are high fluorescence intensity and extremely narrow emission bands. Also, the stains must exhibit a high chemical stability and a fluorescence lifetime compatible with the constraints of the flow system employed. To achieve these diverse features, we plan to design and synthesize a series of rigid, cage-like polychelate complexes containing - as the light emitting center - a trivalent lanthanide: specifically, europium, terbium, samarium, or dysprosium. These chelates will be coupled to proteins through a suitable functional group, and the fluorescence as well as the stability of the protein-bound chelates will be investigated to assess the factors which favor permanent and effective staining.

The proposed research essentially involves the application of the current knowledge and methods in inorganic coordination chemistry, molecular spectroscopy, and organic synthesis to the development of a new tool for cytological investigations.

454. ASPIRATION CYTOLOGY FOR DIAGNOSIS OF MALIGNANCY

Parulkar, G. B., Chitale, A. R., Patel, C. V., Mehta, S. L., King Edward VII Memorial Hospital, Dept. of Surgery, Parel 400012, Bombay, India

The purpose of this study is to emphasize the application of needle aspiration cytology in the diagnosis of clinically detectable tumors of various organs.

A study of 500 cases subjected to needle aspiration cytology was carried out during a period of 3 years at the Surgical Outpatient Department. Cytology slides were prepared, stained

by Hematoxylin and Eosin and results reported immediately. All cytological observations were confirmed subsequently on histology sections. Barring the unsatisfactory samples (62), the accuracy rate was assessed to be 98.8%. There were 5 false negative reports but not a single false positive report given. Out of the 218 malignant tumors diagnosed cytologically, histological sub-typing was not possible in 6 of the cases.

This technique is simple, rapid, accurate and should constitute a routine investigatory procedure in the diagnosis of palpable masses.

455. QUANTITATIVE CYTOCHEMICAL ANALYSES OF NEOPLASTIC AND PRENEOPLASTIC CELL POPULATIONS FOR CYTODIAGNOSTIC PURPOSES

Caspersson, T. O., Karolinska Sjukhuset, Dept. of Tumor Pathology, S10401 Stockholm 60, Sweden

A system of instruments for quantitative cytochemical measurements in cytopathological specimens of various types has been developed. The basic procedures are integrating high resolution microspectrophotometry in ultraviolet and visible spectra, integrating microinterferometry and high resolution fluorimetry. Whole cells and/or cell compartments, which can be optically isolated, are measured in slide preparations. Special arrangements make it possible not only to make one-parameter measurements in cell populations in large series but also to make multiparameter analysis. For the latter purpose the coordinates of the individual cells are semiautomatically measured and stored in a computer memory by aid of which information each individual selected cell can afterwards rapidly be analyzed sequentially in the different instruments.

The present aim of the project is to develop cytochemical supplements to the morphological cytodagnostic techniques, applicable also to clinical problems in tumor diagnosis and treatment.

The following fields are of particular interest: 1) Cytochemical characterization of preneoplastic cells; 2) improved malignancy gradation in different types of tumors; 3) effects of hormones and/or antihormones on tumor cells; 4) improved techniques to distinguish between hyperplastic and neoplastic growth in cases where morphology meets with difficulties.

456. STUDIES OF THERAPEUTIC END RESULTS AND PROGNOSTIC FACTORS

Myers, M. H., Hankey, B. F., Prorok, P. C., Chen, J. S., Heise, H. W., Connelly, R. R., Baranovsky, A., Silverman, D. T., Devesa, S. S., Curtis, R. E., Chen, R., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Biometric Research & Analytic Studies Section, 9000 Rockville Pike, Bethesda, Maryland, 20205, United States of America

Among white cancer patients significant increases in survival over the 10 year span from 1960-63 to 1970-73 were observed for 7 of the 10 most frequently occurring cancers (Lung, Stomach and Pancreas were the exceptions). Among black patients significant increases were noted for cancers of the uterine corpus, uterine cervix, urinary Bladder and prostate. The most remarkable improvements in survival were observed for Acute Lymphocytic Leukemia in Children less than 15. A newly developed age-dependent mathematical model for cancer screening has lead to a more complete understanding of length bias than had previously been possible. Women with lobular carcinoma in situ are at an increased risk of developing subsequent contralateral carcinoma. No elevated risk was observed for intraductal carcinoma. Cervical cancer patients treated by radiotherapy are at an increased risk of developing leukemia and cancers of the lung, bladder, kidney and genital organs. Adjustment of black-white differences in incidence of breast and cervical cancer for socioeconomic levels explained substantial portions of the observed differences for each site. A statistical method utilizing the interval of time between successive diagnoses of cancers has application in monitoring time trends of cancer incidence and for assessment of disease clustering.

457. PLANIMETRIC STUDY OF CONIZED SECTIONS OF CARCINOMA IN SITU

Masi, C. A., Ellena, A. N., Bertoli, R. M., Musacchio, R. A., Inst. Nacional Regional de Oncologia, Gynecology Section, Bv. Zavalva 3399, C.C. No. 366, 3000 Santa Fe, Argentina

Determination of the topography of the lesion in a group of patients treated at the Regional Institute of Oncology. Planimetric study of conized sections from patients cytologically examined in the Regional Institute of Oncology.

458. APPLICATION OF DIGITAL IMAGE PROCESSING TO CYTOLOGY AUTOMATION

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The Contractor requested that several equipment items in the approved budget be deleted and in their place the funds be used to purchase a PDP11/45 computer and associated hardware. It was originally planned to acquire the computer from another funding source which subsequently did not materialize. A supplemental budget request has been submitted for the deleted equipment and additional personnel, which would have accelerated the program. No action was taken on the supplemental budget request as it was felt that it could be handled by in-house methods.

459. ULTRAFAST SCANNER MICROSCOPE IN LABORATORY AUTOMATION

Shack, R. V., Bartels, P. H., Shoemaker, R. L., Durie, B. G., Buchroeder, R. A., Hillman, D. W., University of Arizona, Graduate School, Tucson, Arizona, 85721, United States of America

The objectives of this project are to design, build, and performance test an ultrafast laser scanner microscope capable of acquiring high-resolution image data, at the rate of 60 MHz, for use in automated prescreening for cancer. The fast laser scanner microscope represents a new class of high-resolution microscopes with optics optimized for input into a computer rather than for a human observer. With its data rate of 60 MHz the instrument will be the fastest such device ever designed, and will make it possible to scan a microscope slide area of 2 x 2 cm with 1.6 billion image points within 60 sec. This brings the full information content of high-resolution imagery to bear on the problem of automated cytology at data rates equal to or even greater than those used in flow cytometry, and will make the automated prescreening of cervical and bladder samples feasible once the required special processors are available. The goal for the coming year is to assemble all subsystems of the fast laser scanner and to begin initial performance testing of the system as a whole. This will include tests of the scanner's optical-mechanical performance and of the maintained tolerances. It will include tests of the photometric performance; determinations of effective signal-to-noise ratios and stray light; and operation of the autofocus system under simulated clinical conditions. The scanned images will be displayed and examined for conformance with scanner design specifications.

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